THE AMOEBAE LIVING IN MAN

CLIFFORD DOBELL

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A ZOOLOGICAL MONOGRAPH

BY

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"*Veritas Temporis filia, non Authoritatis.*"
—BACON, *Nov. Org.*

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PREFACE.

I HAVE attempted in the following monograph to give a concise and accurate account of all the amoebae which live in human beings. Although our knowledge of these protozoa has grown apace in the last few years, there is at present—so far as I am aware—no published work which gives a correct description of them from the standpoint of modern protozoology. The works already published—the various treatises on the Protozoa, and innumerable papers scattered through zoological and medical periodicals—reveal, if taken together, a sad state of confusion in the minds of men : and much that is now certainly known appears uncertain, merely because of the conflicting and contradictory statements with which the literature of this subject abounds. That correct solutions of the chief problems of the past, and a true account of the state of knowledge at present, would be welcomed by many zoologists and medical men—especially by those whose work lies in the tropics—I have good reason to believe: and it is in the hope of contributing something towards the attainment of both these ends—the clarification of ideas, and the codification of facts—that the following pages have been written.

This little treatise is addressed to all who are interested in the amoebae of man from a zoological standpoint, and is intended for those who already possess some acquaintance with the science of protozoology. To these it is offered with the hope that the work which the author has expended on it may lighten their labours in the same field. It is, moreover, the work of a biologist and not of a medical man—of one whose chief interest is, and has ever been, in the Protozoa themselves and not in the diseases which they produce—of one whose point of view has been determined not by practice as a physician but by the study of other amoebae and their kindred. Few zoologists have hitherto had an opportunity of studying the amoebae of man, and few medical men have possessed the zoological knowledge necessary for a proper investigation of the material in their hands. What has already been published demonstrates conclusively the need for further work on this subject by protozoologists skilled in all the intricacies of their science ; and if I have presumed to take so great a task upon myself, it is because no other worker has yet volunteered for this difficult but urgent service.

I have devoted a great part of my working life to the study of

amoebae of all sorts, for they have always been with me favourite objects of investigation ; and I probably realize more clearly than most men how very little is really known and understood concerning these remarkable organisms. I confess, indeed, that I still regard them as in many ways the most difficult to investigate of all living animals. No one man will ever succeed in solving all the myriad problems which they present : and it is merely as a small contribution to what must ultimately be the work of many, that these pages are submitted to the judgement of all fellow-workers seeking after truth.

April, 1919. C. D.

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I.

INTRODUCTION.

I FIRST became interested in the amoebae which live in man some twelve years ago. I was then investigating the amoebae of frogs, and the observations which I made led me to study much of the published work dealing with the related rhizopods occurring in man and other animals. At that date Schaudinn's views were generally accepted ; and so great was his prestige, that to question any of his pronouncements in protozoology was almost to stamp oneself—in the estimation of most zoologists and medical men—as an inexperienced or incompetent worker. Nevertheless, my own observations soon constrained me to believe that Schaudinn's conclusions regarding the intestinal amoebae of man were mostly incorrect—as time has since shown convincingly: but although I have always thought that my conclusions concerning the development of the amoebae of frogs would ultimately prove to be equally applicable to the species living in man, I was long unable to verify this owing to my inability to obtain adequate material for a study of the latter organisms.

In the meantime, the wrong road of inquiry opened up by Schaudinn was eagerly explored by many other workers: with the result that our "knowledge" of the intestinal amoebae was soon a muddle of facts, misinterpretations, malobservations, and fanciful speculations, from which—only a few years ago—it seemed almost impossible to extricate the truth.

For some years I followed, with the greatest interest, all the work published on this subject—noting the various "new species" from time to time discovered, reading the writings of the older observers as opportunity occurred, and forming my own opinions of them to the best of my ability. But I very soon realized that my opinions were almost worthless without a very extensive first-hand acquaintance with all the organisms under discussion: and consequently, whilst I criticized and controverted many of the accepted views in conversation, in correspondence, and in my lectures at the Imperial College, I did not permit myself to express any opinions in print. Of this I am now glad, because experience has taught me that it is impossible to draw correct conclusions from merely studying what has been written on this subject. I thought too, and still think, that it is a mistake to confine oneself, in dealing with such a subject, to the parasitic forms; and my own work has, accordingly, been carried out as a part of a more comprehensive study of the amoebae generally—both parasitic and free-living.

The War unexpectedly gave me an opportunity of studying, on a large scale, all the intestinal protozoa of man. In 1915 large bodies of troops from the Eastern War Areas began to arrive in this country. It was at first believed that amoebic dysentery had been responsible for a very large proportion of the invalidism in our troops from Gallipoli and Egypt: and although we now know that this was a mistake, it called for protozoological examination of the stools of a very large number of military patients invalided home with a diagnosis of "dysentery." No machinery for coping with such diagnostic work on a large scale existed at the time, and it thus became necessary to train and organize a number of workers specially for the purpose. This work was begun by Dr. C. M. Wenyon, who was at that time most qualified to undertake such a difficult task; but after he had made a beginning, his services were demanded elsewhere, and at the request of the Medical Research Committee I took charge of the work at the end of 1915.* Since that time I have, with the permission and assistance of the Imperial College, devoted myself uninterruptedly to the practical study of the intestinal protozoa of man. A large part of my time has, of necessity, been occupied with routine work of diagnosis, with teaching, and with the investigation of methods of treating amoebic dysentery. But nevertheless I have had, during the whole of this period, great opportunities for studying the human intestinal protozoa from the zoological standpoint. I believe, indeed, that no zoologist has ever before had such an immense amount of material at his disposal for a study of the intestinal amoebae of man.

During the War I have naturally followed, with the keenest interest, all the work which has been done upon the present subject—so far as the results of other workers, both at home and abroad, have been ascertainable. I have also recently read, or re-read, most of the previously published work, in order to correct my earlier impressions and clear up my former difficulties in the interpretation of the results of many other workers. In the light of present knowledge, and with a large personal experience to fall back upon, I now find but little difficulty in understanding many observations which formerly puzzled me: and so far as the intestinal amoebae of man are concerned, I believe that it is now possible to claim that all the chief problems of the past have been solved. For my own part, at all events, I can now say that almost all my own doubts and difficulties have disappeared.

Having thus reached definite conclusions on many points, and having corrected or verified many of the observations of others, so that their findings are reconcilable with my own, I have thought it a not altogether thankless task to record the main results. I have endeavoured, in the following pages, to deal fairly with the work of all my predecessors; and I have done my best to cope with the immense and scattered literature on amoebae and amoebiasis. I hope I have not omitted to study any previous work of material importance from the zoological standpoint. If so, it is through ignorance of its existence, or inability to gain access to it. On the other hand, I have knowingly ignored much purely medical work; and for the reason that it is not

* A full account of the circumstances here briefly alluded to will be found in the Introduction to Special Report No. 4 (1917), published by the Medical Research Committee.

germane to my present purpose, which is to collect and coördinate all our present zoological knowledge of the amoebae which live in man. My original intention was to confine myself to the intestinal amoebae; but this soon became almost impossible, as the others had so frequently to be discussed or compared. I decided, therefore, to attempt to deal with all the amoeboid organisms which have been described from human beings. My work is obviously incomplete and faulty in many ways, but even these deficiencies may have their uses: for the mere exhibition of a glaring defect, or the clear definition of a difficulty, frequently hastens the advance of science by indicating a pitfall or a wrong road of inquiry.

Although I have always formed my opinions independently, and attach—as every honest worker must—particular importance to my own personal observations, I have undoubtedly been influenced, in the present work, by the advice and assistance which I have received from my fellow-workers. Such help is impossible to assess, or even to define. My obligations in this respect are none the less immense, and I can at least attempt to acknowledge them. In the first place, my indebtedness to Dr. C. M. Wenyon—now Colonel, A.M.S.—is gratefully recorded. When I began to study the amoebae of man in earnest, his help—always most generously given—was invaluable. I learnt more in conversation with him than I had ever previously learnt from books. My ignorance of medicine has to some extent been compensated by working in collaboration with other medical friends, who have always given me help and instruction in the kindest possible manner. In this connexion I am glad of an opportunity to acknowledge my obligations to Dr. H. H. Dale, F.R.S., with whom I had the good fortune to work in the early days; to Dr. A. C. Stevenson, of the Wellcome Bureau, who has shared much arduous work with me throughout, and with whom I have discussed—with much profit to myself—most of the questions here considered; and to Dr. G. C. Low, whose extensive clinical experience has always been freely placed at my disposal. For much good advice and frequent assistance in bacteriological matters I am further indebted to Capt. S. R. Douglas, I.M.S. (ret.) and to the late Dr. H. S. Gettings.

The friends, pupils, and fellow-workers who have helped by giving me material, by supplying me with information, or by directing my attention to various points of interest, are innumerable. But in this connexion I would again thank the following for their assistance on divers occasions: Captain F. W. O'Connor, R.A.M.C., Dr. P. P. Laidlaw of Guy's Hospital, Dr. E. H. Kettle of St. Mary's Hospital, Dr. G. T. Western of the London Hospital; and Messrs. H. A. Baylis, the late W. O. Redman King, A. Malins Smith, J. R. Matthews, Geoffrey Paget, R. E. Savage, A. G. Thacker, and Hugh Watson. Miss M. W. Jepps, who for a time acted as my assistant, has also given me much help. I am further indebted to Captain F. L. Armitage, N.Z.M.C., for specimens from a case of amoebic abscess of the brain; to Dr. J. W. Scott Macfie for material from a case of "urinary amoebiasis"; and to Mr. T. Goodey for some preparations of *Entamoeba gingivalis*. Several patients with interesting amoebic infections have also given me the greatest assistance by submitting themselves to repeated and irksome examination. Though nameless here, they are neither forgotten nor unthanked.

Much of the work whose results are here recorded has been carried

out in London at the Wellcome Bureau of Scientific Research. My best thanks are again offered to the Director, Lieut.-Col. Andrew Balfour, C.B., C.M.G., and all his staff, for their hospitality and unfailing help at all times and on all occasions. The work itself has been done with the aid of grants from the Medical Research Committee, and without this assistance would have been impossible. In addition to this general acknowledgement of my indebtedness to the Committee, I should like to place on record my deep obligation to their Secretary, Sir Walter Fletcher, K.B.E., who not only enabled me in the first place to begin the work, but who also, by constant help and encouragement, insured its accomplishment.

To all those mentioned and to many others my thanks are due and gratefully offered. Nevertheless, I take full responsibility for all the views expressed in the following pages; and if I have fallen into errors, the fault is mine, and not attributable to those who have helped me. In compiling the present memoir I have endeavoured on all occasions to give credit to every worker for his own discoveries, and any omissions in this respect—for such oversights are, I fear, inevitable—are unintentional. I am well aware, moreover, that in a subject of such magnitude and complexity there is little to be said that is really new. My aim has been to set down what is true rather than what is novel. For myself, therefore, I would only say, in the words of that wise old philosopher, John Locke: "Truth needs no recommendation and error is not mended by it; in our inquiry after knowledge, it little concerns us what other men have thought."

II.

A NOTE ON MATERIAL AND METHODS.

I DO not propose to describe in detail all the methods which I have used in studying the amoebae of man. It seems to me unnecessary to do so, because no special or peculiar methods are required for such a study. Any good cytological or protozoological methods of observation, fixation, staining, etc., may be used successfully : and since I have tried most of them, it would be merely wearisome to enumerate them all individually. I wish to say a few words here, however, upon certain points which are of particular importance.

First, I would note that it is impossible to obtain a correct knowledge of the amoebae living in man without studying an abundance of material. The study of only a few infections, or of insufficient material from many, is very apt to lead to error. Secondly, the material must be as fresh as possible, as all the amoebae which live in human beings degenerate and die rapidly outside the body. Most of the mistakes which have been made with regard to the morphology and life-histories of these organisms have been due to failure to take these two elementary precautions : and it is tragic to note, in reading the literature, the vast time and trouble that have been wasted in the study of wholly inadequate quantities of material of the poorest quality. Whatever mistakes I may have made myself have not been due, as a rule, to any lack of material. For a study of the intestinal amoebae I have had more than enough. During the last few years I have personally examined over 10,000 human stools of all sorts : I have also had at my disposal nearly 150 kittens experimentally infected with *E. histolytica :* and I have been able, through the kindness of many friends, to examine a considerable amount of pathological material from cases of amoebic dysentery and other diseases. The obtaining of living amoebae in a really fresh and healthy condition suitable for careful microscopic study—both alive and after treatment by good cytological methods—has not been easy. But the difficulties encountered have mostly been overcome, and I now feel that I have had sufficient experience to enable me to speak with some confidence about the intestinal amoebae at least. My confidence concerning most of the facts recorded in the following pages has been greatly strengthened by the confirmatory observations of a large number of fellow-workers and pupils. The majority of my own observations have, indeed, become the common everyday knowledge—confirmed time after time in the daily routine—of dozens of workers who have been engaged with me in this field of work during the War.

I would note here, however, as a word of warning to many who have studied this subject only from the practical standpoint of diagnosis, that

the methods which suffice for this purpose are not adequate for obtaining a complete knowledge of the amoebae themselves or of their development. For example, if an amoeba found in the stools is so degenerate as to be unidentifiable, its species may, in practice, often be determined by the finding of its cysts. But this does not mean that these amoebae are really undeterminable as to their genus or species; nor does this empirical method permit us to dispense with a study of the normal organisms when considering them from a protozoological standpoint. As a matter of fact, I believe all the species of amoebae living in the human intestine are easily determinable if examined in a fresh and normal state, though often undeterminable under the conditions imposed by laboratory practice. Makeshift methods of examination and determination are only of value for purposes of ordinary laboratory diagnosis, which has often to be made from material which is quite unsuitable for zoological study; and it is hardly necessary to point out that a mere examination of amoebae or cysts in saline or iodine solution—which is usually sufficient for diagnostic purposes—cannot supply that detailed information requisite for a true understanding of the organisms investigated. This information, as I would again emphasize, can be obtained only by studying an abundance of the best material with the aid of all the cytological resources now at the disposal of protozoologists.

The following special points concerning technique may be noted briefly here, as they have some interest or importance.

It is absolutely essential to study the living organisms as well as fixed and stained specimens. Every competent protozoologist now recognizes this as a general principle; but we have already had, nevertheless, numerous descriptions of "amoebae" from man which have never even been seen alive. After studying the living amoebae or cysts, an examination of them in iodine solution* is often very helpful—especially for cysts, in which the iodine-reaction of the contained glycogen often supplies important information. In iodine solution, also, owing to the fixation which takes place, the nuclei become easily visible; and their structure can thus be studied roughly, and their number counted accurately. Any good cytological method of fixation will usually fix amoebae well, but the fixation of cysts is often much more difficult. The best fixative for cysts is, in my experience, Schaudinn's sublimate-alcohol solution to which 4 to 5 per cent. of glacial acetic acid has been added. Cysts—especially those of *E. coli*—are sometimes difficult to stain: and it is useful to remember that they are, in general, more readily permeable to watery than to alcoholic solutions. Grenacher's borax-carmine, used warm, and acidified with a small quantity of glacial acetic or hydrochloric acid, will often stain the contents of cysts when all other methods have failed. For ordinary routine purposes I have found Mayer's haemalum by far the most reliable and rapid stain for cysts of all sorts; though it cannot, of course, replace the finer cytological stains—such as the various iron-haematoxylin methods. My alcoholic iron-haematein

* I do not know who first introduced this method for the study of intestinal amoebae and cysts. The method is, of course, one of the earliest ever used for studying protozoa. McCarrison (1909) Kuenen and Swellengrebel (1913) and others, have used it for studying the cysts of *Entamoebae;* and since its recommendation by Wenyon (1915) as a routine method, it has come into general use. Iodine should be used as a strong aqueous solution in potassium iodide—the stronger the better.

method* is very good for all amoebae, but usually not reliable—owing to unequal penetration—for staining cysts.

I have obtained beautiful preparations of amoebae and their cysts, and of sections of intestinal ulcers and other tissues containing *E. histolytica*, by employing a modified form of Mann's stain. As I have used this method for some years, and taught it to many people, I may mention it here. I use Mann's well-known methylblue-eosin mixture† made up in the usual way, but differentiate the preparations with a very dilute solution of Orange G in 70 per cent. alcohol—instead of using the alcoholic solution of caustic soda which he employs. Very fine results can be obtained by this method—not only with tissues and amoebae, but often with cysts as well. For sections I have also found Borrel's magenta and picro-indigo-carmine method very useful: but a modification of this, in which I use acid fuchsin instead of basic fuchsin, gives even better results with sections containing *E. histolytica*, as the amoebae can be differentially stained by this method. Safranin may also be used instead of magenta, and gives excellent results. For demonstrating glycogen in the cysts of amoebae, Best's specific carmine stain‡ is very useful—both as a control for the reactions obtained with iodine, and as a method by which permanent preparations showing glycogen can be obtained. Cysts may be fixed previously in Schaudinn's sublimate-alcohol solution, or with Carnoy's chloroform-alcohol-acetic-acid mixture: and if it is desired to show the nuclei as well as the glycogen, they can be coloured by previously staining the cysts in Weigert's alcoholic iron-chloride haematoxylin—without removing the glycogen or affecting its staining powers.

All the specimens figured in the Plates have been drawn, with the aid of the camera lucida, at a uniform and exact magnification of 2500 diameters. All the drawings were made from preparations examined under a 2 mm. apochromatic objective (Leitz), with N.A. = 1.40, using compensating oculars and an achromatic aplanatic condenser. The methods by which the specimens figured were fixed and stained are all noted in the descriptions of the Plates. I will only add here that for studying and drawing organisms stained with red stains—such as fuchsin or carmine—I have found it an advantage to use a green light for illumination, as details can then be resolved with greater ease and precision. I have found a Wratten colour screen ("B. Filter," No. 58), placed below the condenser of the microscope, very useful for this purpose. This is, of course, a general method, and not one peculiarly suited to the study of amoebae. I mention it here merely because all specimens actually stained red, but depicted in black in Plates III-V, have been drawn from preparations examined in this manner.

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* *Vide* Dobell (1914).

† *Vide* G. Mann: "*Physiological Histology*" (1902), p. 216.

‡ *Vide* F. Best (1906): *Zeitschr. f. wiss. Mikrosk.*, xxiii, 319.

III.

THE PRESENT STATE OF KNOWLEDGE OF THE AMOEBAE LIVING IN MAN.

BEFORE I attempt to give detailed descriptions of the amoebae which live in man, I will devote a short space to a general account of our knowledge of the whole subject; my object being to indicate, by means of a brief historic survey, the present state of knowledge concerning all the amoebae which live in human beings, and the more important of the steps by which this knowledge has been acquired.

By far the greater part of the literature on this subject deals with the amoebae which live in the intestine. These have always been of particular interest to the physician, on account of their relation—real or supposed—to dysentery and other pathological conditions. But it is only within the last half-dozen years that the medical problems connected with these organisms have been fully elucidated: and it is probable that many more years will yet elapse before the knowledge which we now possess becomes generally current in zoology and medicine. It is a familiar observation that it is often more difficult to establish a truth than to perpetuate an error: and of this the history of the present subject already supplies abundant illustrative instances.

Amoebae appear to have been first found in man by Gros (1849) in Russia, who discovered and briefly described the amoeba now known as *Entamoeba gingivalis*, which lives in the mouth. Steinberg (1862), Grassi (1879*a*), Kartulis (1893), and Prowazek (1904), all rediscovered the same organism later, and in recent years it has acquired some notoriety. The earlier workers appear to have regarded it as a harmless organism: but Bass and Johns (1914, 1915), Smith and Barrett (1915), and other more recent investigators—especially in America—have upheld the hypothesis that it is the cause of pyorrhoea alveolaris, and consequently a pathogenic parasite of considerable medical importance. Much work has since been done on this subject, and it now appears more than probable that the earlier workers—as is so often the case—had the clearer vision. At the present moment all the evidence points to the conclusion that *E. gingivalis* is an inoffensive commensal, which man very commonly, but unsuspectingly, lodges in his mouth. It seems unlikely that the future will reveal any further facts of fundamental importance concerning this amoeba.

It is generally said that the intestinal amoebae of man were discovered by Lambl, in Prague. This is, I think, an error. It is true that Lambl (1860) reported the finding of "amoebae" in the intestine of a child dead of enteritis; but as he also found "*Difflugia*" and "*Arcella*" in the intestinal contents, his observations are open to grave suspicion—as Leuckart (1863) long ago pointed out. His "amoebae," moreover, can hardly have belonged to any of the species now known

to live in the intestine. They measured only 4 μ to 6 μ in diameter, and were almost certainly small degenerating individuals of the flagellate *Trichomonas hominis*. Although this has been pointed out by Leuckart (1863)* and Grassi (1888), modern workers† still continue to cite Lambl as the discoverer of the intestinal amoebae of man.

So far as I have been able to ascertain, the intestinal amoebae of man were really discovered by Lewis (1870) and Cunningham (1871), in the course of their investigations on cholera in India. The amoebae which they studied probably belonged, for the most part, to the species now known as *Entamoeba coli*—as will be shown later. It is therefore not surprising that these workers concluded that the intestinal amoebae of man are not pathogenic. Soon after the publication of their observations, however, another amoeba was discovered by Lösch (1875), in the stools of a patient suffering from dysentery, in Russia: and the discovery of this organism initiated a discussion which subsequently engaged the attention of a large number of workers and lasted for some forty years. The chief point at issue was whether the intestinal amoebae of man do or do not cause dysentery. Opposite views were held by different workers and at different periods—the consensus of opinion swinging first to one side and then to the other.

There appear to me to be two chief reasons why the comparatively simple problems connected with amoebic dysentery remained so long unsolved. First, there was a failure to realize that the amoebae constitute a large group of organisms, containing many species belonging to many different genera. Of these, man harbours not one but several different kinds; and all are forms which have nothing to do with the free-living amoebae. The second obstacle in the way to knowledge was the failure to realize that "dysentery" is not one disease, but a symptom of several different pathological conditions. There is no one specific "cause" of all dysenteric symptoms; and it is now even difficult to conceive how anybody could ever have thought that he had ruled out amoebae, as a cause of dysentery, by simply demonstrating that certain bacteria can also cause it.

We now know that the amoeba which Lösch discovered was *Entamoeba histolytica*—one of several different species living in the human bowel, and the only one, so far as is known, capable of causing dysentery or any other disease in man. We now know also, however, that this organism does not usually cause dysentery, which is most often the result of infection with certain bacteria. At the time of Lösch's discovery bacillary dysentery was not a clearly recognized condition, and all amoebae were regarded—at least by physicians—as suspiciously alike. Lösch himself was not convinced that the amoebae which he found were the cause of his patient's illness, and he seems to have regarded them rather as a secondary or accessory factor in the causation of dysentery. Much of the earlier work on the intestinal amoebae was

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* Leuckart (1863, p. 140) drew attention to the similarity of Lambl's figures—which he reproduced—to an organism described from fowls by Eberth. This organism ("*Trypanosoma*" *eberthi* Kent) is now known, since the work of Martin and Robertson, to be a *Trichomonas*.

† Many of them cite a paper published in the *Vierteljahrschr. f. prakt. Heilkde.*, Prag, 1859. In this, however, amoebae are not even mentioned. I fancy few people have really seen Lambl's work of 1860, which is very difficult to obtain.

done by the Italians—Grassi (1879—1888), Calandruccio (1890), Celli and Fiocca (1894, 1895), Casagrandi and Barbagallo (1895, 1897)—whose observations were in many cases perfectly correct, but unfortunately marred by their failure to recognize the existence of more than one species of amoeba in man. They identified all their amoebae with those of Lösch, whereas they were really those studied by Lewis and Cunningham; and they wrongly concluded, from their investigation of a harmless species, that all intestinal amoebae are equally harmless.

Lösch's amoebae were studied, during the same period, by many other workers. Among these Kartulis (1885—1893), Councilman and Lafleur (1891), Kovács (1892), and Kruse and Pasquale (1894) must be specially mentioned. To Councilman and Lafleur especially belongs the credit of having first stated clearly that there is a particular kind of dysentery caused by amoebae—"amoebic dysentery," as they first called it. Before their time there were workers—such as Kartulis—who apparently believed that "dysentery" in general is invariably and exclusively the result of amoebic infection. Councilman and Lafleur also confirmed the observation of Kartulis (1887) that amoebic dysentery may be followed or accompanied by the formation of hepatic abscesses; and that in such cases amoebae, apparently identical with those found in the stools, may be present in the liver—as Koch* first showed. That "tropical" hepatic abscess is definitely associated with "tropical" dysentery had, however, been recognized long before by the Anglo-Indian clinicians, whose observations thus found their true explanation.

I do not know who first suggested that more than one species of amoeba may inhabit the human bowel. Schuberg (1893) attributes the idea to Kartulis (1891), but it was expressed also by Councilman and Lafleur (1891) and Lutz (1891) at about the same time. Schuberg himself, and Lutz, and most other workers at this period, concluded that there was insufficient evidence to prove the existence of more than one species. Councilman and Lafleur, it is true, believed in the existence of more than one species, but they adduced no evidence in support of their belief. This evidence, however, was promptly supplied by Quincke and Roos (1893); but by one of those curious blunders which so often arrest the progress of science, their observations were almost ignored by their contemporaries, and never received the attention which they merited.

By the year 1897 all the main facts necessary for understanding the relation of amoebae to dysentery had been discovered. It was clear from the work of the bacteriologists† that epidemic dysentery is usually caused by bacteria and not by amoebae. It was equally clear, however, that there is a particular kind of dysentery caused by amoebae—as the work of Lösch, Kartulis, Councilman and Lafleur, Kovács, Kruse and Pasquale, and Quincke and Roos had demonstrated. It was, moreover, evident, from the work of Grassi, Calandruccio, Celli and Fiocca, and Casagrandi and Barbagallo, that all intestinal amoebae do not cause dysentery. Furthermore, it had been shown by Quincke and Roos how the different species of intestinal amoebae can be distinguished from

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* *Vide* Koch and Gaffky (1887).

† Consult especially Janowski (1897) in this connexion.

one another zoologically: and it had been proved, by Casagrandi and Barbagallo, that the intestinal amoebae have nothing to do with the free-living species, from which they differ both morphologically and in the character of being uncultivable in artificial media. It is truly astonishing, in reading the works on the intestinal amoebae of man, such as Behla's (1898), and the larger medical and zoological treatises published at the end of the last and the beginning of the present century, to observe the blindness which appears to have descended upon everybody who studied this subject at this period. Instead of illumination, darkness followed; and the twentieth century began with a period of nearly a dozen years of chaos.

For this period of confusion Schaudinn was, in my judgement, chiefly responsible. Notwithstanding his great services to the science of protozoology in other respects, his influence upon the present subject was almost wholly bad. His work, published in 1903, produced a profound effect, though it was merely a brief preliminary statement of his views—dogmatic, full of errors, unillustrated; and his conclusions, had they been presented by any other worker, would probably not have been accepted without further evidence. There was, indeed, but one fundamental point in which he was not mistaken—his assertion that there are two different amoebae, one pathogenic and the other harmless, inhabiting the human bowel: and nobody who has read the works of earlier observers can give Schaudinn much credit for this "discovery," in which he had been forestalled repeatedly. Nevertheless, the fact remains that when Schaudinn said it, everybody realized its truth; whereas the words of earlier observers fell upon deaf ears. Schaudinn's "life-histories" of the two forms were almost entirely wrong. Some of his observations and experiments are, indeed, so incredible* that it is difficult to believe that they were not sheer inventions. Certain it is, at all events, that no competent worker will ever repeat them. In his revision of the nomenclature of the intestinal amoebae he was equally unfortunate, and for his errors of judgement we still suffer.

Another cause of the arrest of progress in this subject at the beginning of the century was undoubtedly the work of Musgrave and Clegg (1904, 1906), carried out in the Philippines. These workers upheld the thesis that "all amebas are or may become pathogenic." Their chief reason for believing this, apparently, was their inability to distinguish one species of amoeba from another. For them all amoebae were alike. They appear to have been almost uninfluenced by the earlier work of others, and to have thought it unnecessary to study protozoology or cytological methods. Mixing up all species of amoebae indiscriminately, and studying none of them properly, they soon reached the conclusion that "the whole of the surface flora of the Philippine Islands carries a large number of these parasites [*i.e.*, 'amoebae' generally]. Some of which, at least, belong to the class [= species] which produces disease in human beings." The importance they attributed to morphology can be gauged by their statement that "at the present time no value can be attached to it." Upon zoologists generally the expression of such views could naturally produce but

* See, for example, his amazing experiment by which he proved that *E. histolytica* forms minute spores capable of surviving, in a condition infective to cats after complete desiccation. This experiment is, to me, still quite inexplicable.

little effect. Upon medicine, however, Musgrave and Clegg's work has still left its impression; and from time to time others still fall into the same errors. The chief merits of their work consisted in their improvements in the methods of cultivating free-living amoebae, and their introduction of the now current term "amoebiasis," to denote a state of "infection with amebas."

The publications of the first decade of the present century make, for the most part, unpleasant reading. The German workers—Prowazek, Hartmann, and others—continued along the path of error indicated by Schaudinn. Craig, in America, and many others, followed in their train, and their "confirmations" of Schaudinn's work, coupled with the discovery at intervals of new "species" of amoebae in man, served only to make matters worse. A present-day seeker after the truth, completely ignorant of the subject, would obtain more reliable information by consulting the works published prior to 1900 than those which appeared during the next ten years. In the latter period almost the only observation of any real value was made by Huber (1903), but it was stifled by the authority of Schaudinn and the other German workers. Huber rediscovered and first investigated the cysts of the dysentery amoeba—formerly found by Quincke and Roos—and really supplied the chief deficiency in our knowledge of this organism. The rediscovery of these cysts by Viereck (1907), Hartmann (1908), and Elmassian (1909),—who all described them as belonging to new species—did not mend matters. No real advance was effected until the completion of the epoch-making work of E. L. Walker in the Philippine Islands. This work must now be considered.

Walker made an unpromising beginning. His first paper (1908) repeated many of the errors of his predecessors. He was unable to distinguish the free-living from the parasitic species of amoebae, and confused forms which he was able to cultivate, with those found in the intestinal contents of man and other animals. A second paper, published three years later, marked an immense stride forward. Walker (1911) was then able to draw the following conclusions* from his researches:

(1) The amoebae found in the Manila water supply, and those cultivable from the intestinal tract of man, all belong to the genus *Amoeba* Ehrenberg. These species are not parasitic in the intestine of man. When amoebae are obtained in cultures from the intestinal contents, they are derived from ingested cysts which have passed unchanged through the intestine.

(2) The amoebae parasitic in the intestinal tract of man belong to a distinct genus, *Entamoeba* Casagrandi and Barbagallo. They are obligatory parasites, and cannot be cultivated. Two species are recognizable — one non-pathogenic (*Entamoeba coli*) and one pathogenic (*E. histolytica*). The first forms cysts containing 8 nuclei; the second cysts containing 4 nuclei. The organisms are transmitted from man to man by means of these cysts.

In his final paper, published in collaboration with Sellards (1913), Walker was able to supply proofs of all these statements, as a result of

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* I have modified and condensed these conclusions somewhat, so that they are not given here in Walker's own words exactly.

a series of carefully planned and executed experiments on human beings. This work finally solved all the chief problems connected with the relation of amoebae to dysentery. It confirmed and vastly extended the earlier observations of the Italian workers, of Quincke and Roos, and of others, and placed our knowledge of the subject on a firm foundation of fact which is still unshaken and probably unshakable.

There are, however, certain minor details in which Walker's conclusions must now be qualified. His statement that the free-living amoebae belong to the genus *Amoeba* is incorrect. The forms which he studied—the so-called "*limax* amoebae"—certainly do not belong to the genus *Entamoeba;* but it is equally certain that they do not belong to the genus *Amoeba* Ehrenberg, but to other genera. Further, it is now certain that there are not merely two intestinal *Entamoebae* in man. There are, as later work has shown conclusively, at least five different species belong to four distinct genera. Of these, however, only one—*E. histolytica*—is pathogenic, as Walker maintained.

Apart from these later additions to our knowledge, there is little to be changed in Walker's conclusions. His work is, and will probably remain, one of the most brilliant contributions ever made to medical zoology. For my own part, I regard the chief problems connected with the relation of intestinal amoebae to dysentery and other diseases as solved finally by Walker's work. The researches of later workers are, if read in the light of his results, all easily comprehensible and confirmatory. Among these workers Wenyon, Darling, and James, must be specially mentioned, on account of the important additions which they have made to our knowledge: but their work will be considered in greater detail later.

From time to time writers have since relapsed into the old mistakes; but it is clear that this has always been due to ignorance of the facts or failure to understand the knowledge already acquired. In recent years, for example, Gauducheau (1915) has questioned the correctness of Walker's conclusions—but without understanding them, and from the standpoint of the early days of confusion. Again, Knowles and Cole (1917) have counselled us to go back to the same period, by attempting to show that all the intestinal amoebae of man belong to one species. Their proposal to call this hypothetical "species" by the inadmissible name of "*Entamoeba coli communis*" shows how little qualified they are to express any opinion on questions of protozoology, biological systematics, and nomenclature. Many other recent workers have not only been unable—on account of their imperfect zoological knowledge—to distinguish different genera and species from one another, but they still continue to confuse these organisms with cells belonging to the human body. The recent works of Bartlett (1917) and Thomson and Thomson (1916*a*) supply instances of this: but the just criticisms of Bahr and Willmore (1918) fortunately make it superfluous to discuss these observations further. Thomson and Thomson (1916) have even published work undertaken to ascertain whether the dysentery amoeba lives in the sand in Egypt. Their views, apparently, are closely similar to those of some of the earliest workers, and their standpoint is that of Musgrave and Clegg,—which has really been untenable since the days of Casagrandi and Barbagallo, and inexcusable since the work of Walker and Sellards. Many other recent mistakes could easily be cited: but as they all rest upon a similar ignorance of facts which are no longer even

debatable, it is unnecessary to argue about them. Indeed, one can but express surprise when a worker so experienced in other fields as Marchoux (1918) now asks us to reconsider whether there is, after all, such a thing as amoebic dysentery. Opinions such as this can only be regarded as anachronisms, which time will eventually set right.

Although it seems probable that our knowledge of the oral and intestinal amoebae of man is now in a state approaching finality,* this cannot, unfortunately, be said of some of the other amoeboid organisms which have been described from other situations in human beings. There is little doubt, however, that many of these are not amoebae at all, but tissue cells or other bodies mistaken for amoebae. I shall consider some of the more important of these later. It will suffice here merely to notice the possible existence of such organisms.

After this brief introductory survey I shall now pass on to consider in detail the individual species of amoebae which live in man. But before doing so it is necessary to say a few words about the genera to which these species belong—a subject which is by no means free from difficulties owing to the present limited state of knowledge of the amoebae in general.

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* It is, perhaps, not superfluous to point out that although we now possess much exact and probably definitive knowledge of the intestinal amoebae of man, the literature of the subject is still in the greatest confusion. There is not a single text-book, either zoological or medical, which contains an even approximately correct account of these amoebae. Medical works such as those of Brown (1910), Craig (1911), Rogers (1913), and Phillips (1915), on amoebae and amoebiasis, and the zoological treatises of Doflein (1911), Minchin (1912), Brumpt (1913), and others, are compact with errors of all sorts. Inexperienced workers should be specially warned against the work of Rogers (1913), which—though it contains many excellent clinical and other observations—does not contain reliable information concerning the amoebae of man. The figures of "amoebae" in this work are quite unrecognizable, and most misleading.

IV.

THE GENERA OF AMOEBAE LIVING IN MAN.

ALL the older writers who dealt with the amoebae of man placed them in the genus *Amoeba*, which originally included a very heterogeneous collection of naked rhizopods. As knowledge accumulated, however, it became clear that this genus would have to be dismembered; and one of the earliest attempts in this direction was made by Leidy (1879), who proposed to separate the amoeba parasitic in the cockroach (*Amoeba blattae* Bütschli) from the free-living forms. For this organism he proposed the new genus *Endamoeba*.* Consequently, the type species of *Endamoeba* Leidy is *E. blattae* Bütschli.

Casagrandi and Barbagallo (1895 *a*), apparently in ignorance of Leidy's work, proposed a new genus *Entamoeba* for the amoebae which they studied from man. At first (1895 *a*) they called their organism *Entamoeba coli*, believing it to be the same as that described by Lösch (1875) and called by him "Amoeba coli"; but they renamed it later *Entamoeba hominis* (Casagrandi and Barbagallo, 1897).

It is clear from their papers that the actual organism to which these names were applied was the large harmless amoeba of the human colon —now generally known, since the time of Schaudinn (1903), as *Entamoeba coli*. It follows that the type species of the genus *Entamoeba* Casagrandi et Barbagallo is *E. coli*.

The genus *Entamoeba* of Casagrandi and Barbagallo was accepted by Schaudinn (1903) for *E. coli* and also for *E. histolytica*, the dysentery amoeba: and since the appearance of his work it has been customary to refer almost all the parasitic amoebae to this genus. A curious belief seems, indeed, to have grown up that there are but two genera of amoebae—free-living species all belonging to the genus *Amoeba*, and parasitic forms all belonging to *Entamoeba*. Walker (1911, 1913), for example, in his admirable works on the amoebae of man, speaks as though no other genera exist; and most medical writers who have studied amoebae apparently share this belief. No zoologist, however, can now hold such a view for a moment; for it is certain that, from the zoological standpoint, both the free-living and the parasitic species of amoebae belong to many different genera.

There can be little doubt that the two intestinal amoebae of man commonly known as *Entamoeba coli* and *E. histolytica* are co-generic. The characters supplied by their nuclear structure, their cysts, and their development and morphology generally, warrant their inclusion in the

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* Leidy's paper was long overlooked, and his genus forgotten until attention was called to it by Chatton (1910). It may be noted here that Leidy repeated his definition of *Endamoeba* in his large work on the freshwater rhizopods (Leidy, 1879 *a*, footnote p. 300), which also seems to have been generally overlooked.

same genus—whatever that may be. Moreover, the amoeba living in the mouth of man (*E. gingivalis*) cannot at present be distinguished generically from these two forms. But should these three species be placed in the same genus as the amoeba of the cockroach? Should they, in other words, be put in the genus *Endamoeba* Leidy?

At the present moment this question cannot be answered conclusively. Chatton and Lalung-Bonnaire (1912) and Alexeieff (1912) have answered it in the negative, basing their views on the differences in development described by Mercier (1910) in the parasite of the cockroach. It therefore appeared to them necessary to introduce a new generic name for the amoebae of the type of *E. coli*, because these workers—and all others, apparently—consider that "*Endamoeba*" and "*Entamoeba*" are mere variant spellings of the same name. Chatton and Lalung-Bonnaire (1912) accordingly proposed the new generic name *Löschia*, whilst Alexeieff (1912) proposed the new name *Proctamoeba*. Alexeieff's paper was published about a month after that of Chatton and Lalung-Bonnaire; and consequently, when this was pointed out by Chatton (1912), he withdrew his name later (Alexeieff, 1912 *a*), as a synonym of *Löschia*. It would thus appear, at first sight, that the amoeba of the cockroach should be placed in the genus *Endamoeba* Leidy, whilst the three best-known amoebae of man (*E. coli*, *E. histolytica*, *E. gingivalis*) should be placed in the genus *Löschia*.

There are, however, two real difficulties in the way of accepting this solution. First, it is by no means certain, at the present moment, that the amoeba of the cockroach and the amoebae of the *E. coli* type are generically distinct. The distinction was drawn at a time when these amoebae were believed to possess quite dissimilar life-histories. According to Mercier (1910), *E. blattae* has a sexual phase at the beginning of its life-cycle. The cysts liberate broods of large or small amoeboid gametes which conjugate in pairs heterogamously. The previous development within the cysts is simply a process of straightforward nuclear division. On the other hand, according to Schaudinn (1903) and Hartmann (1908) respectively, *E. coli* and *E. histolytica* (then called *E. tetragena*) display a process of autogamy within their cysts; so that a later gamete-formation and conjugation, like those of *E. blattae*, appeared to be excluded from their development. At the present moment, however, the position is very different. We now know, since the work of Walker (1911) and others—which agreed with my original observations (1908, 1909) on the closely related amoeba of the frog—that there is no autogamy in the cysts of *E. coli*, *E. histolytica*, or any of their congeners. The development within the cyst is a straightforward process of nuclear division, like that described by Mercier and others in *E. blattae*. Furthermore, Mercier's account of the sexual process in this species has not yet been confirmed, and the corresponding stages in *E. coli* and *E. histolytica* have never been studied. Whether they are alike or different in this respect has therefore still to be determined; and upon this determination the decision as to whether they should be placed in the same or in different genera will largely depend.

A second difficulty is this. Lühe (1908), believing Schaudinn's (1903) incorrect account of the development of *E. histolytica* to be true, pointed out that this parasite could not be placed in the same genus with *E. coli* and related forms. He therefore proposed the new genus *Poneramoeba*

for *E. histolytica*. Although the grounds for the proposal are now known to have been fallacious, the name was introduced for an easily recognizable organism; and there seems to be nothing in the rules of nomenclature which can render it invalid. Moreover, Lühe's name seems to be the first generic name available for the dysentery amoeba, if it is decided to remove it from the genus *Endamoeba* (= *Entamoeba*); and since *E. coli* and the other harmless forms related to it are co-generic with *E. histolytica*, it follows that all these organisms might have to be placed in the genus *Poneramoeba*. This would be a most unfortunate interpretation of the laws of nomenclature; for it would place all the harmless species, which constitute the greater part of the group, in a genus designed for, and designatory of,* the one exceptional species which is known to be pathogenic.

Chatton and Lalung-Bonnaire (1912), believing *E. histolytica* to be subgenerically distinct from the amoebae of the *E. coli* type, proposed a new subgenus *Viereckia* to contain it. According to their nomenclature the organism should be called *Löschia* (*Viereckia*) *tetragena*. From Chatton's later publications it may be gathered that he has abandoned this view, for he now calls the dysentery amoeba *Entamoeba dysenteriae*. The name *Viereckia* appears, in any case, to be a synonym of *Poneramoeba*—if the dysentery amoeba is to be placed in a genus apart. At the moment, however, there seem no adequate grounds for separating it from other amoebae such as *E. coli*, *E. muris*, or *E. ranarum*.

In my opinion it is most undesirable to change the names of the amoebae living in man unless this course becomes absolutely necessary. I propose, therefore, provisionally to retain the name *Entamoeba* Casagrandi et Barbagallo, 1895, for the organism to which it was given—namely, *E. coli*—and for all those species which are clearly co-generic (*E. histolytica*, *E. gingivalis*, *E. muris*, etc.); and to retain also the genus *Endamoeba* Leidy, 1879, for the one organism—*E. blattae*—for which it was proposed. It may be argued that *Endamoeba* and *Entamoeba* are different spellings of the same name, or that they differ too slightly from one another to be kept separate. This has already been urged by many writers, and is doubtless justifiable. Nevertheless, nobody can say at present whether the organisms originally called *Endamoeba* and *Entamoeba* respectively are generically the same or different: and if the difference is at present so slight and uncertain, then a slight difference between their generic designations might not inappropriately express it. Whether ultimately shown to be right or wrong, this course is, I think, the one which will give rise to the least confusion at present. I cannot, for my own part, accept with equanimity any drastic change in nomenclature which will certainly lead to confusion—however plausible a case may be made for it by those who care more for the "correctness" of names than for the codification of knowledge. What, for example, should we gain by calling the dysentery amoeba, which every worker in England has known for years as *Entamoeba histolytica*, by the new name *Poneramoeba coli*? And yet a very plausible case indeed can be made out for this combination.

I shall therefore continue to refer three of the common amoebae of man—namely, *E. coli*, *E. histolytica*, *E. gingivalis*—to the genus

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* *Poneramoeba*, from πονηρός, causing pain, harmful.

Entamoeba Casagrandi et Barbagallo, 1895; whilst provisionally I reserve the separate genus *Endamoeba* Leidy, 1879, for the amoeba of the cockroach. On this system, the type species of *Entamoeba* is *E. coli*, and the type of *Endamoeba* is *E. blattae*. They have not one common type.

I may perhaps remark here that although it is clearly incorrect to attribute to any authority a name which he did not employ, this has frequently been done in the case of the names under discussion.* For example, I note that Craig (1912, 1912 *a*) writes "*Entameba* Casagrandi and Barbagallo"; later (Craig, 1913 *b*, 1914) "*Entamoeba* Leidy"; still later (Craig, 1917) "*Endameba* Leidy." Not one of these is the name employed by the authority cited. Moreover, I would protest against the suppression of the diphthongs in *Amoeba*, *Entamoeba*, and *Endamoeba*, which has now become habitual with most American writers. They may be justified in translating the English word "amoeba" into the American "ameba" (plural, "amebas"): but it is difficult to see what grounds they can have for altering the Latin language—for generic names like "*Entamoeba*" are, theoretically at least, Latin and not American. For my part, I find "*Endameba*" almost as unpleasant as the quite unjustifiable and offensive "*Entamöba*" which German writers frequently employ. Obviously, "*Amoeba*" (and all derivatives such as *Entamoeba* or *Endamoeba*) is orthographically correct as a generic name only when written thus in its original form.

In addition to the three amoebae belonging to the genus *Entamoeba* there are three other species which have to be noted here. First, there is the organism named *Entamoeba nana* by Wenyon and O'Connor (1917). This organism clearly belongs to a different genus from that typified by *E. coli*. From examination of the evidence I consider that it should be placed in the genus *Endolimax* Kuenen et Swellengrebel (1917). This question will be considered in greater detail, however, in the discussion of the nomenclature of this species. (*Vide infra*, p. 101.) Secondly, there is the peculiar binucleate amoeba for which—in a joint paper†—I have proposed the name *Dientamoeba*. The nomenclature of this organism has already been discussed in detail in the earlier paper. Thirdly, there is another intestinal organism which is described in the present work, but which has previously been known in a disconnected and incomplete manner. This is the organism called *Entamoeba bütschlii* by Prowazek (1912 *a*), but whose cysts were called "Iodine cysts" by Wenyon (1916). As this amoeba cannot be placed in any of the existent genera, I shall propose the new genus *Iodamoeba* for it. The nomenclature of this organism will be discussed later, in the description of the species.

In the next section I shall attempt to give a systematic account of all the species of amoebae from man. Before doing so, however, I may summarize the conclusions reached in this section. They are set forth in the following synopsis, which will show the genera, species, and types at a glance, and will also serve as a key to the species described in

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* In addition to the instances cited I may also note that Hartmann (1913) writes "*Entamoeba* Leidy emend. Schaudinn"—a remarkable combination when it is remembered that Leidy's real name (*Endamoeba*) was unknown to Schaudinn, who used *Entamoeba* Casagrandi et Barbagallo.

† *Vide* Jepps and Dobell (1918).

the ensuing sections. It should be added that the doubtful organisms discussed in a later section (p. 134) are purposely excluded from this synopsis, as our knowledge of them is still too uncertain to allow of their classification—supposing them to be amoebae. It is probable, however, that many of them are not even protozoa.

Synopsis of Genera and Species of Amoebae living in Man.

Genus I. *Entamoeba* Casagrandi & Barbagallo, 1895.
(nec *Endamoeba* Leidy, 1879.)

Synonyms :
Poneramoeba Lühe, 1908.
Löschia, *Viereckia* } Chatton & Lalung-Bonnaire, 1912.
Proctamoeba Alexeieff, 1912.
[*Amoeba* (*pro parte*), *Endamoeba*, *Entameba*, *Endameba*, *Entamöba*, Auctt.]

Type : *E. coli* (Grassi) Casagrandi & Barbagallo.

Species in Man : *E. coli* (Grassi) Casagrandi & Barbagallo.
E. histolytica Schaudinn (*emend.* Walker).
E. gingivalis (Gros) Brumpt.

Genus II. *Endolimax* Kuenen & Swellengrebel, 1917.
Only species, hence type : *E. nana* (Wenyon & O'Connor) Brug.

Genus III. *Iodamoeba* nov. gen.
Only species, hence type : *I. bütschlii* (Prowazek) Dobell.

Genus IV. *Dientamoeba* Jepps & Dobell, 1918.
Only species, hence type : *D. fragilis* Jepps & Dobell.

V.

GENUS *ENTAMOEBA* CASAGRANDI & BARBAGALLO, 1895.

THREE species belonging to this genus are found in man. I shall begin with the most important—

(1) *ENTAMOEBA HISTOLYTICA* SCHAUDINN, 1903 (*EMEND.* WALKER, 1911).

"Amoeba coli" Lösch, 1875.
? "Amoeba urogenitalis" Baelz, 1883.
? *Amoeba vaginalis* Blanchard, 1885.
? *Amoeba intestinalis* Blanchard, 1885.
"Amoeba dysenteriae" Councilman & Lafleur, 1891.
Amoeba coli (Lösch) } Kovács, 1892.
Amoeba dysenteriae (Councilman & Lafleur) }
"Amoeba coli felis" Quincke & Roos, 1893.
Amoeba lobosa var. *coli* Celli & Fiocca, 1894.
Entamoeba histolytica Schaudinn, 1903.
Entamoeba dysenteriae (Councilman & Lafleur) Craig, 1905.
[*Entamoeba*] "*tetragona*" (Schaudinn) Huber, 1906.
Entamoeba coli var. *tetragena* Viereck, 1907.
Entamoeba africana Hartmann, 1907.
Entamoeba tetragena (Viereck) Hartmann, 1908.
"Entamoeba Schaudinni" Lesage, 1908.
Poneramoeba histolytica Lühe, 1908.
Entamoeba minuta Elmassian, 1909.
Entamoeba nipponica Koidzumi, 1909 (*pro parte*).
Entamoeba hartmanni Prowazek, 1912.
Entamoeba coli Werner, 1912 (*pro parte*).
Löschia (*Viereckia*) *tetragena* Chatton & Lalung-Bonnaire, 1912.
Entamoeba brasiliensis Aragão, 1912 (*pro parte*).
Löschia histolytica (Schaudinn) Mathis, 1913.
Entamoeba venaticum Darling, 1915.
Entamoeba minuta Woodcock & Penfold, 1916 (*nec* Elmassian, 1909).
"Non-pathogenic *E. tetragena*" Shimura, 1916 (*pro parte*).
Endamoeba coli (Lösch) Aragão, 1917.
Endamoeba dysenteriae (Councilman & Lafleur) Pestana, 1917.
Entamoeba tenuis Kuenen & Swellengrebel, 1917.
Entamoeba minutissima Brug, 1917.
Endamoeba histolytica (Schaudinn) Craig, 1917.
Entamoeba coli communis Knowles & Cole, 1917 (*pro parte*).

HISTORIC.

Entamoeba histolytica, the dysentery amoeba of man, was discovered by Lösch in 1873 (*vide* Lösch, 1875), in the stools of a young Russian peasant named Markoff, who had come, from his village in the Govern-

ment of Archangel, to look for work in Petrograd. Here he contracted dysentery, and after spending about 5 months in hospital, died from an intercurrent attack of pneumonia. Lösch has left descriptions of the patient, the parasites, and the *post mortem* findings, which leave no room for any doubts concerning the interpretation of his case.

Lösch's patient suffered from a persistently relapsing dysentery, with bloody mucous stools in which large numbers of very active amoebae were often present. They measured, when rounded, 20-30 μ as a rule, though sometimes more ; and "not seldom" they contained red blood corpuscles, and occasionally leucocytes and fragments of epithelial cells. Their ectoplasm was clearly differentiated from their endoplasm, and each amoeba possessed a vesicular nucleus with a well-defined membrane and a minute central nucleolus—points which are all illustrated by good figures. Attempts were made to infect 4 dogs, by mouth and rectum, with fresh stools containing the parasites. One dog contracted dysentery, with numerous amoebae in its evacuations, and was ultimately killed. A *post mortem* examination showed that its large intestine was ulcerated, amoebae being present in the ulcers and in the intestinal contents. When the patient died, the autopsy revealed a similar condition of ulceration in his large intestine. Lösch considered that his amoebae were different from any previously described, and proposed to call them "Amoeba coli." Though he remained in doubt as to the precise relation of the parasites to the patient's disorder, he appears to have believed that they were not the primary cause of the dysentery, but acted rather as mechanical irritants which prevented the healing of the dysenteric ulcers originally caused by some other agency.

Lösch studied another case of amoebic dysentery later at Kieff. It was recorded by Massiutin (1889), who described four other cases of amoebic infection—all probably (?) infections with *E. coli*—and who also considered that amoebae do not directly cause dysentery. The "*Amoeba coli*" found by Grassi (1879 *a*), and studied by him and other Italian workers subsequently, was—in all probability—wrongly identified with Lösch's amoeba. It was, for the most part, *Entamoeba coli*—not *E. histolytica*.

The next discovery of importance, after Lösch's observations, was made by Koch in 1883, though not published until a few years later (Koch and Gaffky, 1887). In conducting inquiries for the Cholera Commission sent to Egypt and India in 1883, Koch had occasion to make *post mortem* examinations of 5 cases of dysentery—2 of them complicated with liver abscess. Sections of the intestinal ulcers of 4 of these cases revealed "peculiar amoeboid structures," of variable shape and "about 1½-2 times as large as white corpuscles." They were present in sections only, or in material from the bases of the ulcers—never in the dejecta or gut contents : and in one case they were also present in the capillaries round the wall of the liver abscess. Koch appears to have regarded these bodies as "amoebae," but his observations, at the time, were by no means unequivocal. There can be little doubt now, however, that he actually observed *E. histolytica*, apparently for the first time, in the primary lesions in the bowel and also in its secondary site of infection in the liver.

Further observations upon the occurrence of amoebae in dysentery were soon recorded in Egypt by Kartulis (1885, *et seq.*). In his first paper (1885) he described, from 6 cases, "giant amoebae ?" which do

not appear to have been amoebae at all. What they were I cannot determine from his account.* In a second paper, however, published in the following year (Kartulis, 1886), he gives a very different description of "amoebae." He says he studied 150 cases of undoubted dysentery in Egypt, and found amoebae in all. In sections of the intestinal ulcers of 12 of these cases he also succeeded in finding the parasites—thus confirming the observations of Koch. Control cases, not suffering from dysentery, were never found infected. The amoebae are said to have measured 12-30 μ in diameter, and other characters are also noted; but his description of them is very inferior to Lösch's. At this date Kartulis could record only negative results from his attempts to infect laboratory animals, and to cultivate the parasites. He considered, however, that the amoebae were the cause of "tropical dysentery," and appears to have identified them with Lösch's "Amoeba coli." Although it is now certain that many of Kartulis's amoebae really were *E. histolytica*, it is inconceivable that he could really have found this parasite in every one of 150 cases of clinical dysentery examined in Egypt, and in no non-dysenteric cases. Some at least of his dysenteric patients must have been cases of bacillary dysentery, and it would be impossible now for any competent worker to examine many non-dysenteric persons in Egypt without finding *E. histolytica*.†

A year later Kartulis (1887) published another important paper, announcing that he had found his amoebae in the pus of liver abscesses—thus confirming the much earlier suspicions of the Anglo-Indian doctors, and the observation of Koch, that "tropical" liver abscess is a sequel to a certain form of dysentery and due to the same cause. A fuller account of his observations was published two years later (Kartulis, 1889); and still later he recorded that he had been able to infect cats with the amoebae, and thereby to produce dysentery in them experimentally (Kartulis, 1891). In this he appears to have been anticipated by Hlava (1887), working at Prague. Unfortunately, Kartulis (1891) also claimed to have cultivated the dysentery amoebae (in straw infusions, exposed to the air), and to have produced dysentery in cats by injecting the cultures. These obviously fallacious experiments were soon seized upon and discredited by other workers, and served rather to weaken than to strengthen his contention that intestinal amoebae are the cause of "tropical" dysentery and liver abscess. The last important contribution made by Kartulis to our knowledge of the dysentery amoeba was his discovery of the parasite in abscesses of the brain (Kartulis, 1904). That the brain, like the liver, may be secondarily infected is now a well established fact—foreshadowed long ago in the work of Morehead and the Anglo-Indian clinicians, and fully confirmed by Legrand (1912) and others.

The discovery of amoebae in post-dysenteric liver abscesses was soon confirmed by Osler (1890) in America, whose observations led Councilman and Lafleur (1891) to undertake an extensive investigation into the pathology of "amoebic dysentery" and "amoebic abscess of the liver"

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* Although called "giant" forms, their size is given as "0·00015—0·000222 mm." but the figures—stated to be magnified about 100 diameters—show much larger bodies. No structure can be made out in them, and none is described. It is not stated that the "amoebae" were motile when alive.

† Compare, for example, the findings of Wenyon and O'Connor (1917).

—terms which they introduced. Although their work is still a classic from the standpoint of pathology, it added almost nothing to our knowledge of the amoebae concerned. From a zoological point of view their account of *E. histolytica* is greatly inferior to Lösch's.

Kovács (1892) studied 2 cases of amoebic dysentery, and experimentally infected 5 kittens with the amoebae, of which he gave a clear description. There can be no doubt that he studied *E. histolytica*, and that his work was an important confirmation of the earlier observations. He observed typical intestinal lesions in his cats, but failed to cultivate the amoebae. Fuller confirmation of the facts discovered by Lösch, Koch, Kartulis, and Councilman and Lafleur, was published soon afterwards by Kruse and Pasquale (1894), as a result of investigations carried out in Egypt. An important new point which they* brought to ligh was the fact that the amoebae in liver abscess pus—which is bacterio logically sterile, as Kartulis (1887) first showed—are able, if injected *per rectum*, to infect a cat and give it dysentery. They successfully performed this experiment three times out of seven attempts.

The experiment clearly indicated two important conclusions: namely, that the amoebae associated with dysentery are identical with those found in the pus of liver abscesses, and that the parasites are causally connected with these diseases. A counterpart to this experiment was furnished later by Harris (1901), who succeeded in infecting puppies with the amoebae from a human case of dysentery. Not only did they acquire amoebic dysentery—as in Lösch's (1875) experiment—but two of them also developed amoebic liver abscesses subsequently. At the same time it was shown that the bacteria cultivated from the stools of the patient did not cause dysentery when introduced into the dog's intestine. Several workers have since produced amoebic liver abscesses experimentally in cats by a similar procedure.†

The most important zoological work at this early period, however, is that of Quincke and Roos (1893) and Roos (1894), carried out in Kiel; for it not only showed that more than one species of amoeba inhabits the human bowel, but it also showed with equal clearness how these species may be differentiated, and how man becomes infected with them. It is astonishing that this fundamentally important work has hitherto received so little attention.

Although Quincke and Roos studied only a single case of amoebic dysentery, they studied it very carefully; and they controlled their observations by a study of the amoebae occurring in non-dysenteric cases. The amoebae found in the patient suffering from dysentery are well described, and recognizably figured.‡ The sharp demarcation between the ectoplasm and the endoplasm, the appearance of the nucleus, the

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* Schuberg (1893) states that this experiment was first performed by Kartulis (1891). This author says, however, that he cultivated the amoebae from a hepatic abscess—*in pure culture*, free from all bacteria: and with this culture he claimed to have infected a kitten, which acquired typical amoebic dysentery and showed typical ulceration of its gut *post mortem*. In the light of our present knowledge the interpretation of this result by no means free from difficulties.

† Craig (1905), Huber (1909), Wenyon (1912), Baetjer and Sellards (1914*a*), Dale and Dobell (1917).

‡ By Roos (1894). The woodcuts in the first communication by Quincke and Roos (1893), are very crude.

frequent presence of red corpuscles in the cytoplasm, and the striking activity of the organism, are all noted. In their account of all these characters Quincke and Roos confirmed the observations of Lösch, Kovács, and other early workers. But in addition, they discovered the cysts of the parasite in the stools of their patient. They are described as rounded, refractile structures with a thin but definite wall, smaller than the active amoebae, and measuring 10-12 μ in diameter. By means of careful experiments they proved that a cat can be infected, and acquire amoebic dysentery with characteristic intestinal lesions, by causing it to swallow the cysts, or by injection of the active amoebae *per anum*. All these characters were emphasized as distinctive of the amoebae associated with dysentery in man : for they found that the other amoebae which they studied (in reality *E. coli*) differed in all the characters noted. They were sluggish, contained ingested foreign bodies, but never red corpuscles, formed larger cysts (16-17 μ) with a thicker wall, and were non-infective and non-pathogenic for cats. From their observations they drew the conclusion that man acquires his infection with the dysentery amoeba by swallowing its cysts —as they had shown to be possible in the case of the cat. The only thing of any importance that Quincke and Roos failed to do was to investigate the cytological details of the amoebae and their cysts. They merely noted that the latter contain "nucleus-like structures," but they did not study these properly nor count them. Roos's figures show cysts with one or two nuclei (indistinct in some), and indications of chromatoid bodies. In the matter of nomenclature they were unfortunate; for though they rightly identified their pathogenic amoeba with Lösch's "Amoeba coli," they wrongly proposed to change its name to "Amoeba coli felis," on account of its pathogenicity for the cat.

The dysentery amoeba was restudied and redescribed by many workers in the following decade, but nothing material was added to our knowledge of it. Jürgens (1902) confirmed the earlier observations on the amoebae, and Schaudinn (1903) renamed them. But neither of them studied the cysts again, or understood the life-history of the organism. Schaudinn, indeed, added a wholly incorrect account of its development, far behind that of Quincke and Roos. He failed to find the cysts, and substituted a highly imaginative account of "sporulation" in place of encystation.*

In the very same year that Schaudinn's erroneous statements made their appearance, a real discovery was made by Huber (1903) : but so great was the authority of Schaudinn, that Huber's work was—and is—almost completely ignored. Huber (1903) confirmed the observations of Quincke and Roos. He studied a typical case of amoebic dysentery, he saw the amoebae and their cysts, and he infected cats with the former *per rectum* and with the latter *per os*. He added the important observation that the cysts contain 1, 2, or 4 nuclei, but never more, and also chromatin masses and blocks, and can thus be distinguished from the cysts of the

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* According to Schaudinn, *E. histolytica* does not encyst but forms resistant spores, 3-7 μ in diameter. These are described as being formed by a kind of "budding,' which is now generally supposed to have been a degenerative fragmentation. Craig (1908) "confirmed" this account, and published figures of the stages: but notwithstanding the circumstantial evidence brought forward by both these workers, I am unable to decide what these "spores" really were. Craig has since recanted, but he has not wholly explained his previous findings.

"ordinary" amoeba (*i.e.*, *E. coli*, as described by Schaudinn). Huber told Schaudinn* of his observations—which were perfectly correct—but neither the latter nor anybody else who knew of them seems to have attached any importance to them at the time. After Schaudinn's death the cysts were once more "discovered" by Viereck (1907) and by Hartmann (1907),† who regarded them as belonging to new species of *Entamoeba*—named by them respectively *E. tetragena* and *E. africana*. Elmassian (1909) again "discovered" them two years later, and regarded them—together with the precystic amoebae which form them—as another new species, which he named *E. minuta*. These various "discoveries," and others made during this period, only served to add to the existing confusion. Quincke and Roos's observations were forgotten, Huber's were ignored, everybody looked for—and some found—the non-existent development of *E. histolytica* described by Schaudinn. No real advance in our knowledge of the dysentery amoeba took place until the work of Walker (1911) made its appearance, followed soon after by his later memoir, in collaboration with Sellards (1913), which solved most of the problems connected with *E. histolytica*.

Walker (1911) first showed that *E. histolytica* and *E. coli* are quite distinct and easily separable species, though possessing a similar development. The first forms cysts containing, when mature, 4 nuclei; the second cysts containing 8 nuclei. In the cysts of both, development occurs in a straight-forward manner by the repeated division of an originally single nucleus—without any "autogamy" or other mysterious processes such as were described by Schaudinn. Then he showed that *E. histolytica*, *E. tetragena*, and *E. minuta* are all different names for one and the same species. Finally, with Sellards (1913), he proved conclusively by experiments on human beings that man becomes infected by ingesting the cysts of these amoebae; and that infection with *E. histolytica* may give rise to dysentery, while *E. coli* is harmless to its host. To Walker (1911, 1913) we also owe the conception no less than the discovery of the "carrier" condition in *E. histolytica* infections—a conception which cleared up all the difficulties which previously prevented the life-history and activities of this organism from being properly understood. Certain details of Walker's work will be considered later. Here the historic importance of the work as a whole is all that immediately concerns us.

All the correct observations made since the appearance of the results of Walker and Sellards merely confirm or elucidate the facts which they established. It will therefore be unnecessary to consider, at this point, all the minor details contributed by numerous subsequent workers; but I would especially mention here the names of Darling (1912, *et seq.*), Wenyon (1912 *et seq.*), and James (1914), who have all made valuable later contributions to our knowledge of *E. histolytica*.

Nomenclature.

The nomenclature of the dysentery amoeba has been for some time a very vexed question. It has already been discussed *ad nauseam* by numerous writers: and my only excuse for reopening the question is my desire to reach finality in the matter. I have already discussed the

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* *Vide* Huber (1906, 1909).

† *Vide* Hartmann and Prowazek (1907).

nomenclature of this organism briefly elsewhere (Dobell, 1918), and will begin by recapitulating what I there said.

The dysentery amoeba was first described by Lösch (1875), who named it "Amoeba coli." Consequently, if his name is accepted, and the parasite is placed in the genus *Entamoeba*, its correct name—according to the rule of priority—is *Entamoeba coli* Lösch. This name, however, was most unfortunately assigned to the large harmless amoeba of the human colon by Schaudinn (1903), in his revision of these forms : and since then it has been used with no other signification. To transfer this name now can only lead to the direst confusion. So far as I am aware only one writer has hitherto had the temerity to advocate such a course—Aragão (1917, 1917 *a*), who considers that we should henceforth call the dysentery amoeba *E. coli*, notwithstanding the confusion it will create, in order to conform to the law of priority. Many other workers* now call the dysentery amoeba *E. dysenteriae*†, on the grounds that this specific name was given to it by Councilman and Lafleur in 1891, and therefore has priority over *histolytica* Schaudinn (1903). This course was first recommended by Craig (1905), who abandoned it later when Stiles (1905) showed that it was not justifiable. Stiles's revision of "Amoeba coli" is, however, no longer acceptable, because he did not know all the facts of the case.‡ He showed, nevertheless, that *dysenteriae* Councilman and Lafleur, 1891, is not available as a name for the dysentery amoeba—as I have also pointed out (1918) in ignorance that Stiles had already done so. Councilman and Lafleur proposed to call Lösch's "Amoeba coli" by the new name "amoeba dysenteriae" solely because they considered the former inappropriate.§ Their name is therefore a synonym of "Amoeba coli" Lösch, if these names are considered to have any standing. For my own part I consider "amoeba dysenteriae" to be unquestionably synonymous with "Amoeba coli" Lösch ; but I also regard it as having no systematic status whatever. It was written in ordinary type, without a capital letter for the generic name ; and, moreover, as the context shows, it was proposed as a descriptive term and not as a binominal Linnaean name. On no grounds, apparently, can *E. dysenteriae* be justified as the name of the dysentery amoeba.

The singular point in the nomenclature of those who call the dysentery amoeba *E. dysenteriae*, is that they all, with few exceptions, give the name *E. coli* to the species to which Schaudinn gave it.‖ This curious inconsistency I have already pointed out (1918). It appears

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* For example Brumpt (1913), Mathis and Mercier (1916), and many other workers in France and America.

† Kartulis (1893) and numerous medical writers have used the term "Amoeba dysenterica"—presumably in mistake for "*dysenteriae*."

‡ Stiles (1905) concluded that if there is only one amoeba in man, its proper name is *E. coli* Lösch ; but if there are two—a pathogenic and a non-pathogenic—then their names are respectively *E. histolytica* and *E. coli*, as determined by Schaudinn.

§ "We have called the organism, which was first described by Lösch under the name of amoeba coli, the 'amoeba dysenteriae.' The name given to it by Lösch is not distinctive . . etc." Councilman and Lafleur (1891), p. 405.

‖ Pestana (1917) is the only exception I can recall. He names the dysentery amoeba *E. dysenteriae* and the non-pathogenic species *E. hominis*—which abolishes the name *coli* altogether, though with no apparent justification.

to be quite unjustifiable. If Lösch's name "coli" is accepted at all, it must, according to the rules, be given to the dysentery amoeba, and to no other: and it is not permissible to use its synonym "dysenteriae" to replace it, and then to bestow "coli" upon another species. One way out of this difficulty has recently been suggested by Mesnil (1918), who thinks that Lösch originally gave the name *Amoeba coli* to a mixture of species — one of which was later called *dysenteriae* by Councilman and Lafleur. Unfortunately there is no evidence that this was the case. There is absolutely nothing to indicate that Lösch studied more than one species, and to my mind there can be no doubt as to which this was—namely, the dysentery amoeba. Schaudinn (1903), it is true, was "unable to decide" whether Lösch studied the pathogenic or the harmless species, and consequently gave his name to the latter: but no experienced modern worker can share his doubts.

Schaudinn (1903) displayed a singular lack of judgement in his revision of the name "Amoeba coli." Beyond a doubt he should have called the dysentery amoeba *Entamoeba coli* Lösch and the non-pathogenic species *E. hominis* Casagrandi et Barbagallo. This would have created no confusion at the time, as people were then accustomed to call the dysentery amoeba *Amoeba coli*.* Since Schaudinn made his mistake, however, his names have been almost universally adopted; and they should now, in my opinion, be preserved at all costs. I believe this can be done, moreover, without violation of the Rules of Zoological Nomenclature, though it appeared to me impossible when I last discussed the problem (1918). I now offer the following solution:

"Amoeba coli" Lösch (1875) may be regarded as a descriptive term, and not a binominal Linnaean name within the meaning of the code. It was introduced in the following words: "Da die von mir beschriebene Amöbe, so viel mir bewusst, überhaupt mit keiner der bisher bekannten Formen vollkommen übereinstimmt, so scheint es mir gerechtfertigt, dieselbe bis auf Weiteres mit einem besonderen Namen zu bezeichnen und nach ihrem Fundorte etwa Amoeba coli zu nennen" (Lösch, 1875, p. 208). The generic name is written with a capital—as it would be, in any case, in German—but in ordinary type. There is nothing to indicate that Lösch did not employ it as a mere descriptive term†—in the customary medical manner (*i.e.*, like the ordinary names of bacteriology, or Councilman and Lafleur's "amoeba dysenteriae"). Undoubtedly many of the earlier workers regarded it in this light (*e.g.*, Councilman and Lafleur). The name *Amoeba coli* written in italic type, and as a proper zoological name, seems to have been first used by Grassi: and the organism to which he gave it was probably the non-pathogenic form—not the dysentery amoeba—owing to a misidentification. Consequently, I regard "Amoeba coli" Lösch, 1875, as a synonym of *E. histolytica* Schaudinn, 1903, but not a valid zoological name; and *Amoeba coli* Grassi, 1879, as the first valid name given to the organism which Schaudinn (1903) later called *Entamoeba coli*—as

* Musgrave and Clegg (1904) say that the amoebae in human stools were "usually" called *Amoeba dysenteriae* when large and containing red corpuscles, and *Amoeba coli* when smaller, devoid of blood corpuscles, and believed to be non-pathogenic. The earlier literature does not bear out this statement.

† And with some hesitation, as the word "etwa" before it seems to imply.

Casagrandi and Barbagallo (1895) had done before him. I regard "amoeba dysenteriae" Councilman and Lafleur, 1891, as ruled out because it is a synonym of "Amoeba coli" Lösch, and in addition not a valid name, but a descriptive term. Similarly the name "Amoeba coli felis," employed for the same organism by Quincke and Roos (1893), is not a valid Linnaean name, but a trinominal descriptive phrase.* This name has no status in zoological nomenclature. There are three other possible early names for the dysentery amoeba—"Amoeba urogenitalis" Baelz, 1883, *Amoeba vaginalis* Blanchard, 1885, and *Amoeba intestinalis* Blanchard, 1885. The first two of these depend upon the identification of the amoebae found in human urine, which is such a large subject that I shall devote a separate section to it (*vide infra*, p. 125). I conclude there, however, that neither of these names should be employed. The other name—*A. intestinalis* Blanchard—cannot properly be used for any organism, I think. Blanchard (1885) introduced it for some amoebae which are stated by Leuckart (1879) to have been seen by Sonsino in the stools of a child suffering from dysentery. They are merely stated to have been 8-10 times the size of a red blood corpuscle and therefore apparently larger than Lösch's amoebae. They may have been the dysentery amoeba, but they are not identifiable.

Whilst it is true that the terms *A. coli* and *A. dysenteriae* were sometimes used correctly as zoological names, yet they were never used with clear specific conceptions before the time of Schaudinn. We constantly find the name "*A. coli*" used indiscriminately for two different species. It ought not to have been—after Quincke and Roos, Kovács, and Casagrandi and Barbagallo—but nevertheless it was. Thus we find even so competent a zoologist as Doflein (1901) describing "*A. coli* Lösch" as the one and only amoeba from the human intestine —illustrated by Lösch's figures of the free forms of *E. histolytica* and Grassi's figure of the cyst of *E. coli*. I therefore regard Schaudinn's name for the dysentery amoeba as the first proper zoological designation† of the species; and I shall, accordingly, continue to call this organism *Entamoeba histolytica* Schaudinn, 1903. Whether right or wrong, this name is the only one which can now be used without creating chaos in the nomenclature of the amoebae of man.

The chief synonyms of *E. histolytica* are given in the list which heads this section. A few explanations may be given here, however, in justification of the inclusion of certain names.

Amoeba lobosa var. *coli* is the name proposed by Celli and Fiocca (1894*a*), in accordance with their system of nomenclature, for Lösch's amoeba. It is therefore a synonym of *E. histolytica*. "*Amoeba lobosa*" is not a proper name for any amoeba, and Lösch's organism cannot therefore be classified as a variety of a non-existent species. "Entamoeba Schaudinni" Lesage (1908) is presumably a name intended, by this

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* Like "Bacillus coli communis" and many other bacteriological names which are clearly not formed in accordance with the rules of nomenclature.

† Though his description is not, of course, by any means the first. Schaudinn himself considered that the best account was that of Jürgens (1902)—who did not name the amoebae, and later (Jürgens, 1903) adopted Schaudinn's nomenclature. Schaudinn ignored or underrated the work of many of his predecessors—*e.g.*, Kovács, Roos, etc.

author, to denote *E. histolytica*. Consequently it becomes a synonym of the latter. *Entamoeba nipponica* Koidzumi (1909) almost certainly included* *E. histolytica* and possibly also *E. coli* and other amoebae (?) and tissue cells. Darling (1913), by misquotation, renamed it *E. nipponensis*. *E. hartmanni* Prowazek (1912 *a*) was certainly for the most part *E. histolytica*—a strain producing small cysts. *E. tenuis* Kuenen and Swellengrebel (1917) and *E. minutissima* Brug (1917) are similar strains of *E. histolytica*. Swellengrebel (1917) has described these small cysts as belonging to the flagellate *Chilomastix mesnili:* Aragão (1912) has included them with cysts of *E. coli* in his "*E. brasiliensis*": Woodcock and Penfold (1916) call them *E. minuta*, though this is not the *E. minuta* of Elmassian (1909), which was the common strain with cysts about 12 μ in diameter. Everybody will admit now that *E. tetragena* and *E. africana* Hartmann (1907) are synonyms of *E. histolytica*. Nevertheless, a word may be said here about the former, as it has a curious and little known history.

From the papers of Huber (1903, 1906, 1909) it is clear that he rediscovered the cysts of *E. histolytica* at about the time when Schaudinn (1903) named this parasite and described its "spore-formation." Huber showed the cysts to Schaudinn, who would not admit that they belonged to *E. histolytica*: but he told Huber that they belonged to a different species, which he had studied in one case himself, and which he was going to call *E. tetragena*. By a misprint in the original version (Huber, 1906), this name is written "*tetragona*"—a mistake† which Huber (1909) corrected later. When Viereck (1907) rediscovered the cysts, he tacitly adopted Schaudinn's name, and followed him in regarding the species as distinct from *E. histolytica*. However, he appears to have considered that it was really a variety of *E. coli*, and proposed *tetragena* as a varietal and not as a specific name. Hartmann (1908), after rediscovering "*E. africana*," decided that it was identical with Viereck's species, and named it *E. tetragena* Viereck—still supposing it to be a new species. The muddle thus created by the German workers, as a result of ignoring Huber's work and supporting Schaudinn's wrong observations and interpretations, has survived in certain quarters to the present day. *E. tetragena* and *E. histolytica* are names of the same organism, and there is no justification whatever for employing the former. Nevertheless, Kuenen and Swellengrebel (1913) and some other modern workers refuse to give it up. A variant on the name (*E. "tetragina"*) was introduced by Walker (1911), through a mistake of some sort. The sooner all these names—*tetragena*, *tetragona*, *tetragina*—are forgotten, the better will it be for both zoology and medicine.

It remains to add that the "amoeba" described by Noc (1909) from cases of dysentery was partly *E. histolytica*. His forms from the stools, containing red blood corpuscles, undoubtedly belonged to this species: but the forms which he cultivated were just as certainly free-living amoebae, and not *E. histolytica*. The "*E. histolytica*" which Lesage (1905) cultivated from dysenteric stools, and with which he performed some

* Some of the forms of "*E. nipponica*" are stated to have contained red blood corpuscles. If these really were amoebae—and not endothelial cells—then they must have been *E. histolytica*.

† At first sight this looks like a pun on Viereck's own name—but it seems to have been unintentional.

incredible experiments on cats (Lesage, 1907 *a*), were similar free-living forms. The "Amoeba II" of McCarrison (1909), regarded by him as possibly *E. histolytica*, was clearly in reality a cell and not an amoeba. The "non-pathogenic *Entamoeba tetragena*" of Shimura (1916, 1918) is, apparently, chiefly *E. histolytica* from healthy carriers. The "*E. coli*" of Werner (1912) apparently included *E. histolytica* also—to judge from his figs. 32-38, Pl. II. And it is not improbable that the smallest amoebae of "*E. williamsi*" described by Prowazek (1911, fig. 19) were the precystic amoebae belonging to a strain of *E. histolytica* which forms small cysts. But it would take too long to note all the names given to *E. histolytica* by numerous authors who have casually included a few individuals or cysts of this species in their descriptions or figures of others. Specially noteworthy instances will be mentioned later.

There are still two groups of amoebae which have to be taken into account in discussing the nomenclature of *E. histolytica*; namely, the amoebae found in human urine, and those found in the intestines of various animals—especially dogs and monkeys. These will be considered later (see p. 125 *et seq.*), as they cannot be conveniently discussed here: but I will forestall the conclusions there reached by noting at this point that I find sufficient justification for the inclusion of the names "Amoeba urogenitalis" Baelz (1883), *Amoeba vaginalis* Blanchard (1885), and *Entamoeba venaticum* Darling (1915), in the list of synonyms of *E. histolytica*. On the other hand, I find as yet insufficient evidence for the inclusion of any of the names given to the amoebae of monkeys.

Entamoebae in many ways closely similar to *E. histolytica* have also been found in several other animals. These, too, have been named, and the synonymy of the dysentery amoeba cannot be completed without taking them into account.

Entamoeba ranarum (Grassi) Dobell occurs in frogs and toads (cf. my papers 1908, 1909, 1909 *a*). It resembles *E. histolytica* so closely in certain ways as to suggest that it may be the same species. The precystic amoebae and the cysts of the two forms are sometimes indistinguishable. Their identity was suggested by Alexeieff (1914), and appeared to merit further inquiry. Experiments which I made in 1916 seem, however, to show clearly that *E. histolytica* and *E. ranarum* are distinct species. At all events, it was found impossible to infect tadpoles with the cysts of *E. histolytica*. (*Vide* Dobell, 1918.) Another amoeba whose cysts closely resemble those of *E. histolytica* is *E. aulastomi* Nöller (1912), which lives in the hind-gut of a leech,* *Aulastomum gulo* Moq.-Tandon (= *Haemopis sanguisuga* L.). At present there is no proof that this amoeba is not identical with *E. histolytica* or *E. ranarum*, though it will probably, I think, turn out to be a distinct species.

Conclusions regarding Nomenclature of the Dysentery Amoeba.

The conclusions which I draw from the facts noted in the foregoing paragraphs may be summed up briefly as follows:

> The name used for the dysentery amoeba of man should be *Entamoeba histolytica* Schaudinn, 1903, as this is the first zoological name correctly given to this species, and the only one

* Not of "the eagle," as Craig (1913*b*, 1914, 1917) states—apparently as a result of mistranslation of the German word "Egel."

which can now be used without creating confusion. The names *Entamoeba coli* or *E. dysenteriae* should in no case be employed. The former can be justified, from the standpoint of nomenclature, but not from that of common sense. The latter name has no status in zoological nomenclature.

A full summary of the nomenclature and synonyms of this species is provided in the list of names which heads this section (p. 20).

Description.

Entamoeba histolytica has been so frequently described that I shall confine my description to its chief characters, and shall then discuss several points of importance on which there is not yet a general agreement among the various workers who have already described the parasite.

The active forms of *E. histolytica* show great variation in size, ranging from about 18 μ in diameter up to about 40 μ. As a rule, however, they measure between 20 μ and 30 μ when rounded. The living animals, when fresh and healthy, are extremely active. They flow along in a slug-like manner with great rapidity, and show no conspicuous differentiation between ectoplasm and endoplasm. When they have been outside the body of their host for some time, however, at a temperature lower than that of the body, they present quite different though equally characteristic movements. They then remain in one place, throwing out large, hyaline, blade-like pseudopodia composed of ectoplasm sharply separated from the endoplasm. This movement is, in my opinion, seen only in animals which are already in some degree degenerate. But it is very characteristic of this species, and serves to distinguish it from *E. coli*.

The endoplasm of the parasite contains numerous small granules (microsomes), which may be easily stained *intra vitam* with neutral red,* flavine, and several other dyes. Apart from these granular constituents it is homogeneous and colourless, resembling ground glass in optical texture. The cytoplasm as a whole is characterized by its clearness and freedom from inclusions. Degenerate amoebae often contain bubble-like vacuoles, but these are never present in healthy individuals. They begin to appear soon after the amoebae have left the body. Food vacuoles may or may not be present. When present they contain red blood corpuscles, and occasionally leucocytes and fragments of other cells. Normally they do not contain, in my experience, any other inclusions.† Red corpuscles may be present in very large numbers. I have seen an amoeba in which I could count over 40. As a rule, however, they are not so numerous—1 to 10 being most commonly seen. These ingested red corpuscles are, of course, a very characteristic feature of this species: and it is probably safe to assume that any amoeba

* It is not true, as Cutler and Williamson (1917) appear to believe, that *E. histolytica* is the only intestinal amoeba of man which stains with neutral red *intra vitam*. A few simple experiments with other species will suffice to show the fallacy of this view.

† Wenyon and O'Connor (1917) describe and figure (Text fig. 4) specimens of *E. histolytica* containing spores of a bacillus (*B. megatherium* ?) which they had apparently ingested.

found in a human stool is *E. histolytica* if red corpuscles are present in its protoplasm. (Cf. Wenyon and O'Connor, 1917.) As the corpuscles are digested in the food vacuoles, they usually appear more or less eroded or fragmented when inside the amoebae, and distinctly smaller than those seen in the blood or in bloody stools. Contractile vacuoles are, of course, invariably absent—as in all *Entamoebae*.

The nucleus of *E. histolytica* is very characteristic. It has the general structure typical of the genus *Entamoeba*, but contains less chromatin than that of most other species. In the living animal it is inconspicuous or invisible, but becomes prominent and distinct as the organism dies. It is vesicular and usually spherical, measuring about 4 μ to about 7 μ in fixed and stained specimens. Its structure can be satisfactorily studied only in amoebae which have just left the body; for example, those which have just been passed *per anum*, or which have just been scraped out of an intestinal ulcer or other amoebic lesion. The nuclei of nearly all the amoebae seen in a dysenteric stool, examined in the usual way, are generally degenerate,—to a greater or less extent; and most of the descriptions and figures of the nuclei of *E. histolytica* hitherto published appear to be based largely upon such degenerate individuals.

The normal nucleus, when properly fixed and stained, always shows the same structure. (See figs. 1, Pl. I and 16, Pl. II.) It has a very delicate achromatic membrane externally,* which is lined usually by a single layer of small chromatin granules—thus giving the nucleus an annular appearance in optical section. As a rule the granules are of very uniform size, and are either in contact or only very slightly separated from one another. The centre of the nucleus is occupied by a small spherical karyosome, about 0·5 μ in diameter, composed, in all probability, entirely of chromatin. I find no evidence that it contains a centriole—as is so frequently alleged. The most carefully fixed and stained preparations, examined with the best optical apparatus, invariably show the karyosomes in all healthy normal individuals to be quite homogeneous. A most important point about the karyosome is its position in the nucleus. In typical healthy individuals it lies at the centre. An eccentric karyosome is exceptional. (Cf. figs. 1, 16.)

In addition to the chromatic karyosome just described there is frequently a more or less definite achromatic capsule-like structure which surrounds it, giving to the whole the appearance of a deeply stained central granule surrounded by a paler halo. This structure is perhaps best seen in specimens stained by Mann's method (cf. fig. 1, Pl. I), in which the karyosome itself is stained red, and the "halo" blue. In some individuals which otherwise appear perfectly normal this appearance cannot be clearly seen, and I am still undecided as to its correct interpretation. I think the halo should be regarded as a part of the karyosome; but it is possibly merely a coagulum formed by fixation and deposited on the chromatic part, which represents the true karyosome. In my view the karyosome is probably a composite structure, consisting of a central core of chromatin surrounded by a more delicate cortex—the "halo"—of achromatic substance. This

* As stated by Walker (1911), though previously denied by Schaudinn (1903). The achromatic membrane can often be easily seen in badly fixed specimens in which the chromatin granules have been artificially separated from it.

interpretation is supported by comparison with *E. coli*, in which the cortical layer is more conspicuous.

The area between the karyosome and the peripheral layer of chromatin granules is normally free from chromatin. In fixed and stained individuals it appears to be filled with a network of linin, often with definite threads radiating from the karyosome (see figs. 1, 16). This "network" I regard as the optical appearance of an alveolar structure probably formed by fixation. In degenerate or badly fixed individuals, and in deeply-stained specimens, "chromatin" granules may sometimes be seen in the linin network. But these are always, I believe, artifacts or chromatin fragments or granules detached from the periphery or from the karyosome.

The nuclear characters just enumerated—the uniform layer of small chromatin granules at the periphery, the central karyosome with its halo, and the absence of chromatin in the intervening space—serve to distinguish *E. histolytica* with certainty from other intestinal species of the genus *Entamoeba*. Unfortunately, one has often, in practice, to make a diagnosis from organisms which are largely degenerate, and in which the nuclei are abnormal; and as it is frequently impossible in such cases to determine the species of a given amoeba from the structure of its nucleus, the red corpuscles in the cytoplasm often afford a more serviceable specific criterion of *E. histolytica*.

Some writers have laid considerable emphasis on the position which the nucleus occupies in the organism. It is frequently stated* that in the genus *Entamoeba* the nucleus is eccentric,† whereas in the genus *Amoeba* and other free-living forms it is central. It is true that the nucleus in most entamoebae is not centrally placed: but it is not true that the nucleus is usually central in species of the genus *Amoeba*. The belief appears to me to have originated from the fact that the small free-living amoebae—commonly but incorrectly referred to the genus *Amoeba*, and wrongly called collectively "*Amoeba limax*"—usually have more or less centrally placed nuclei when fixed and stained. In these amoebae, however, as in all others, the nucleus constantly changes its position with the movements of the organism: and it is, consequently, no more typically central in them than in other forms. The central position of the nucleus in a fixed and rounded small organism is, I think, an artifact, which can be explained in a purely mechanical manner. It depends merely upon the size of the organism and the physical state of its protoplasm whether its nucleus is or is not centralized in the process of fixation. At all events, in large amoebae—of any genus—this phenomenon is not seen.

Many other descriptions have already been given of the nucleus of *E. histolytica*. There are also plenty of figures already published showing blocks and irregular masses of chromatin on the nuclear membrane, chromatin in the achromatic zone, and broken up karyosomes. Even parasites without karyosomes have been described and figured. That

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* For example, see Phillips (1915): "The Entamoebae have an eccentric nucleus in the resting organism instead of a centrally placed one" (p. 8): whereas in the "genus Amoeba (Ehrenberg) . . . the nucleus in the resting organisms . . . is in a more or less central position" (p. 6).

† This was, I believe, first emphasized—in the case of the intestinal amoebae of man—by Schaudinn (1903).

these descriptions are all based upon defective and insufficient material anybody can easily convince himself, if he will take the trouble to obtain really fresh and healthy amoebae, and study them with sufficient care and with proper cytological technique. All the abnormal forms of nuclei so often described as normal can be made at will by simply keeping normal individuals until they degenerate and die.

Hartmann (1908, *et alibi*) lays great stress upon the occurrence of "cyclical changes around the karyosome" in *E. histolytica*. In normal individuals, however, these are never seen. His "cycle" represents an arbitrary series of degenerate forms with various arrangements of chromatin between the karyosome and the nuclear membrane. All these forms are common in stale stools or liver-abscess pus. But they are not encountered in really fresh and well-fixed material, in which the nuclear structure is constantly as I have just described it.

The differences so often described between the nuclear structure of *E. histolytica* and "*E. tetragena*" are clearly due to misunderstandings of various sorts, since these names are synonymous. I do not understand the views of those who still—whilst admitting the synonymy—distinguish between "*histolytica*" and "*tetragena*" forms of this amoeba. The distinction is, in any case, quite unjustifiable.

General Outline of the Life-history.—The life-history of *E. histolytica* in man is very simple. The active amoebae just described live in the tissues of the gut wall, where they multiply by division. In a typical "normal" infection, a certain proportion of the amoebae constantly leave the ulcers and pass into the lumen of the large bowel, where they encyst and later pass out, in the encysted form, with the faeces. The precystic amoebae, which are thus free in the lumen of the bowel, are smaller than the ordinary forms which continue to multiply in its tissues. They are entirely free from cytoplasmic inclusions, and are in all probability formed simply by division of the larger tissue-inhabiting individuals. The cysts, which are found only in the faeces, are slightly smaller than the precystic amoebae, and when fully developed are quadrinucleate. They vary greatly in size; and it has been shown that there are at least several distinct races of the parasite distinguishable by the size of the cysts which they form. When the cysts are swallowed by a human being, they probably hatch in the small intestine and liberate small amoebae, which pass down with the intestinal contents into the large bowel. Here they attack and invade the tissues, and thus begin the cycle of development anew. No other stages are known to occur normally in the life-history. In exceptional circumstances, however, a complication in development may result from the migration of the amoebae from their primary site of infection into the liver, brain, or other organs. If they succeed in establishing themselves in these secondary sites they cause abscesses, which may give rise to further pathological complications. The amoebae in secondary infections of the organs are always of the typical tissue-inhabiting form—precystic amoebae and cysts being found in the intestinal contents only. Such secondary infections are clearly accidents in the life-history of the amoeba. They do not form a part of its normal cycle of development, but must be regarded as side-issues resulting from the straying of certain individuals from their normal or primary habitat, which is undoubtedly the wall of the large intestine. However important such migrations may be for man from a medical point of view, they are of no use to the

amoebae; since the erring individuals are unable to complete their development, and are thus inevitably lost to the species as a whole.

Other life-histories of *E. histolytica* have been described, but they appear to rest upon misinterpretations of various sorts. The more important of these will be considered later. I would note particularly here, however, that there is no "spore-formation" such as Schaudinn (1903) described in this species, and that "schizogony," or multiple fission, does not occur; and further, that no conjugation or other sexual process has yet been shown to take place at any stage in the life-history.

The chief points in the habits and life-history, as outlined above, will now be considered in further detail.

Habitat.—The normal habitat of *E. histolytica*, as already noted, is the tissue of the large intestine* of man. The parasites are here found in the mucous, submucous, and occasionally the muscular layers, where they cause a typical ulceration. I need not here discuss the morbid anatomy of the various forms of intestinal amoebiasis. Good descriptions are to be found in the publications of Councilman and Lafleur (1891), Dopter (1905, 1907), Kuenen (1909), Christoffersen (1917), and others, to whose works the reader may be referred.

The commonest secondary site of infection is the liver, where the parasites give rise to the formation of abscesses. There can be little doubt that they reach this organ by way of the portal vein. From the liver they may pass in the blood stream to the lungs, brain, and possibly other organs, where they may also settle down and cause the formation of abscesses. Amoebic abscess of the brain is a very uncommon disease, only about 50 cases having been recorded.† Amoebic abscesses in the spleen have been described by J. P. Maxwell (1909) and Rogers (1913), and infections of the urinary system may occur.‡ I have studied several cases of liver abscess and specimens from one cerebral abscess—thanks to my friend Capt. L. Armitage, N.Z.M.C.—and I can confirm the observations of many other workers that the amoebae in these situations are indistinguishable from the forms in the gut wall. The pathological anatomy of these various lesions does not concern us here.

E. histolytica has been stated by Lesage (1907) to occur at times in the blood; in the skin, by various workers (cf. p. 140); and in other situations. The evidence for such statements is, however, still far from convincing. Job and Hirtzmann (1919) have recently alleged that *E. histolytica* ("*Amoeba dysenteriae*") is intracellular during its youngest stages of development in the liver. But their account is too brief and unconvincing to overthrow all the observations of other workers—none of whom have ever found truly intracellular stages of this parasite.

Mode of Nutrition.—A study of sections of intestinal ulcers and liver abscesses, and of the amoebae themselves as they occur in fresh dysenteric stools, makes it abundantly clear that *E. histolytica* nourishes itself at the expense of the tissues. The amoebae penetrate the tissues by destroying the cells. In all probability they do not force their way mechanically into the healthy tissues, but secrete a powerful cytolytic

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* Infection of the small intestine by *E. histolytica* is extremely rare. It has been described by Harris (1898) and Kuenen (1909) in man, and I have seen two instances in the cat (cf. Dale and Dobell, 1917).

† See especially Legrand (1912), Sitsen (1913), and Armitage (1919).

‡ Cf. p. 125 *et seq.*

ferment which first dissolves them. Schaudinn's name "*histolytica*" is most appropriate for this parasite; but his description of the amoebae forcing their way through the tissues and dislocating the cells by means of their tough and powerful pseudopodia appears to be unfounded.* Good sections show clearly that the amoebae apply themselves to the tissues, which then break down; and the organisms thus come to lie in pools of histolysed tissue which they evidently absorb as nutriment. As already noted, they may also ingest red blood corpuscles or fragments of cells, but this is probably the exception rather than the rule. Their chief food is, in all probability, derived from the destroyed tissues, but it is absorbed and not bodily ingested in the manner typical of amoebae. In acute amoebic dysentery, when much blood is present in the intestinal contents, a large proportion of the amoebae passed in the stools may contain red corpuscles: but in sections of intestinal ulcers the proportion is, in my experience, considerably less.

A belief that *E. histolytica* depends, in some mysterious way, upon certain bacteria with which it lives in "symbiosis," is continually encountered in the literature. It appears to have arisen at a time when it was believed—from the work of Kartulis, Celli and Fiocca, and others—that the amoebae cultivated from stools were identical with the dysentery amoeba: and it has been maintained by Musgrave and Clegg (1904) and more recent workers apparently for a similar reason. It is true that the small free-living amoebae can only be cultivated together with bacteria; but this, of course, is because these micro-organisms form the food upon which such amoebae live. There is here no question of a "symbiosis," in the proper sense of the word. Moreover, there is no evidence whatsoever that *E. histolytica* depends for its existence upon bacteria of any sort. It does not eat bacteria, like the free-living amoebae, and no concrete evidence has ever been brought forward to show that it lives in symbiosis—properly so called—with any other organism.† The hypothesis at present adds an unnecessary complication to the life-history of the parasite, and is not worth discussion until some facts can be produced in its favour.

Pathogenesis.—Only since the publication of the admirable work of Walker (1911, 1913), has the relation of *E. histolytica* to man been correctly understood. All the more recent work—including the vast experience gained during the War—has abundantly confirmed his conclusions.‡ It is thus possible, I believe, to deal now with these, and their consequences, as facts; and to give them here with that brevity which certain knowledge alone will permit.

E. histolytica, although a tissue parasite, does not usually cause dysentery or any other clinical symptoms in its host. As it destroys the tissues, these regenerate, and the parasite and its host live in a state

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* Schaudinn appears to have taken this notion from Jürgens (1902). It has found favour also with Craig and others.

† The suggestion that attacks of amoebic dysentery are due to some unexplained co-ordinated action between *E. histolytica* and certain unknown bacteria, appears to be equally unfounded. The idea has been frequently expressed, however, since the time of Janowski (1897), though on what grounds I am unable to discover. It originated, I believe, through a confusion of amoebic with bacillary dysentery.

‡ The important work of Wenyon and O'Connor (1917) requires special mention in this connexion.

of equilibrium.* This is the "normal" or most usual condition, and an infected individual in such a state of equilibrium is called—following Walker (1911, 1913)—a "carrier" of the parasite. He can only be distinguished from uninfected individuals by the presence of the cysts of *E. histolytica* in his stools. His large bowel is ulcerated, more or less, but this is not visible externally and gives rise to no clinical symptoms.

When the parasites and their host do not live in harmony with one another—as happens in a certain proportion of cases—pathological conditions result. These affect both the host and the parasite. In the case of the former, they are manifested as various diseases, which are of three main types: (1) Irritation of the intestine, producing most commonly diarrhoea and intestinal irregularities of divers sorts, and leading, in severe cases, to a typical form of dysentery (Amoebic Dysentery). (2) Generalized effects resulting from the destruction of the lining of the bowel, but not manifested as local intestinal diseases (General Amoebiasis). (3) Secondary disorders consequent upon the wandering of the parasites from the gut into other organs, such as the liver, where they give rise to inflammatory and suppurative conditions (Amoebic Hepatitis, Hepatic and Cerebral Abscess, etc.). All these diseased conditions of man are harmful to the parasite also, for they disturb its food supply, interrupt its normal life-history, and lead to a great wastage and mortality among the amoebae concerned. In amoebic dysentery, for example, the amoebae are cast out of the body in large numbers before they can encyst; and they consequently perish and are unable to propagate their species. Similarly, in the case of secondary infections of the organs, the parasites may enjoy a brief spell of reproductive activity; but they do not encyst in any situation save the gut, and are, in the organs, cut off from the outside world with no means of continuing their race. The various amoebic diseases are thus "diseases" for the parasites as much as they are for their hosts. And it is clear that infection with *E. histolytica* cannot invariably, or even usually, be accompanied by acute dysentery; for if it were the parasite would soon be exterminated.

Entamoeba histolytica is thus a pathogenic parasite in a restricted sense. It is always a destroyer of tissue, but by no means always productive of disease. The usual type of human infection is that exemplified by the carrier of the parasite. The carrier state will therefore be considered here rather more fully, as it involves several matters of importance.

Carriers.—The carrier of *E. histolytica* was first defined by Walker (1911, 1913). It is true that others had previously spoken of amoebic carriers, but they used the term in a different sense, and without comprehending the facts upon which Walker's conception rests. For example Martini (1908) and Vincent (1909) both described and discussed "carriers" of *E. histolytica;* but they were ignorant of the life-history of the amoeba, and of the part played by its cysts. They apparently regarded "carriers" merely from the clinical point of view, and on analogy with the carriers of various bacillary infections. Walker's conception of the carrier of *E. histolytica* is, however, in many ways different: his "carrier" is, in fact, something *sui generis*—by no means

*I have already elaborated this conception somewhat in an earlier publication Dobell, 1918 *a*).

exactly comparable with the bacillary "carriers" of various sorts. It is, perhaps, unfortunate that the same term should have been employed for both; but since it is in current use, it seems inadvisable to try to change it now.

We can now see that the carrier of *E. histolytica* is merely the ordinary individual in the normal state of infection. He is the individual who is naturally adapted to his parasites, and who suffers no appreciable harm from their presence. From the diagnostic point of view, he is the individual who passes the cysts of the amoeba in his stools. If the amoebae do him no harm, and find his bowel a comfortable environment in consequence, they develop in their normal manner —completing their life-cycle by encystation. The carrier of *E. histolytica* can therefore be accurately defined as the individual who passes cysts of the parasite in his stools.

The carrier obviously "carries" the active amoebae in his tissues. He is a carrier of amoebae. He is not properly called a "cyst-carrier" —a term which has unfortunately been introduced into many languages, and which is now used by many workers—because he does not "carry" cysts* in any ordinary sense. He deposits cysts as soon as they are formed. Nobody would call a man infected with an intestinal worm an "egg-carrier": because if he "carries" anything, it is clearly the worm, —not the eggs which it lays and which he discharges in his stools. "Cyst-carrier" is similarly a misnomer, and does not correctly represent the facts.

Walker (1913) divides carriers into two classes—*contact carriers* and *convalescent carriers*. The former are those people who have never suffered from amoebic dysentery; the latter, those who have had an attack of amoebic dysentery, but who have then recovered clinically without losing their infections. On clinical grounds it is important to recognize these two categories; for the contact carrier is typically a healthy individual, whose infection does him no appreciable harm, while the convalescent carrier is the individual who has shown himself susceptible to the action of the parasite. He has already suffered, and frequently continues to suffer, from their presence. Clinically, he is often a case of relapsing dysentery, with intermissions of variable duration when he passes into the carrier state. There is, of course, no hard and fast line between the typically healthy carrier and the patient suffering from acute amoebic dysentery. They are the extreme manifestations of one common condition—intestinal amoebiasis—connected by all intermediate states, any of which may be seen in different individuals or in the same individual at different times.

Carriers are of importance from two different standpoints: the practical, because they alone are the source of infection to others; the theoretical, because they explain all the apparent contradictions which previously prevented people from understanding the part which *E. histolytica* plays in the causation of amoebic dysentery. It is now easy to understand—though unfortunately still not generally understood—how

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* We even read, in recent papers, of carriers of *E. histolytica* being "infected with cysts." This shows a most incomprehensible ignorance of the true conditions. It is doubtless due to the same confusion of ideas that leads people to talk of "cyst-carriers," and to ask for methods of treatment that will enable them to "kill the cysts" inside an infected person.

it is that *E. histolytica* is the cause of amoebic dysentery and other diseases, and yet usually "non-pathogenic": how amoebic diseases are not contracted from persons actually suffering from them: and consequently, how amoebic dysentery and liver abscess come to be endemic and never epidemic in their incidence. For every abnormal individual suffering from dysentery, but non-infective to others, there are dozens of comparatively healthy infected individuals—carriers—who show no clinical signs of infection, but whose amoebae undergo their normal development, and whose cyst-containing faeces are infective to others.

There can be little doubt that *E. histolytica*, even when it causes no dysenteric or other recognizable symptoms, must always live at the expense of its host's tissues. Every healthy carrier has the lining of his large bowel more or less ulcerated; though the ulceration may be, and probably often is, superficial and almost invisible *post mortem* to the naked eye.* But even quite extensive ulceration may exist without any dysenteric symptoms being evident. This is clearly shown by the *post mortem* findings of Musgrave (1910), Bartlett (1917), and others; and it is undoubtedly incorrect to suppose—as many still do—that because an infected person does not suffer from dysentery his intestine is therefore not ulcerated. A point of importance in this connexion is the fact that a person may suffer from an amoebic abscess of the liver or other organ without ever suffering from dysentery. Now *E. histolytica* reaches the liver by way of the portal vein. To get into this the amoebae must traverse the wall of the gut, and to do so they must damage the tissues. Consequently, the parasites must have caused at least some ulceration of the intestine before they gained access to the liver. The case described by Armitage (1919) is particularly interesting† in this respect: for the patient was always, so far as his intestinal infection was concerned, a contact carrier of *E. histolytica*, who had never suffered from dysentery or other intestinal trouble. But he acquired a typical amoebic abscess of the liver, and when this was almost cured, an amoebic abscess of the brain, from which he died. In his stools the cysts of the parasite were present, and in the abscesses the typical tissue-inhabiting amoebae. Such cases as this show clearly that the contact carrier is not infected with a non-pathogenic strain of amoebae—as some would argue; and also that ulceration of the intestine may be present in the absence of all dysenteric symptoms.

Walker (1913), Wenyon and O'Connor (1917), and most other competent observers appear to share these views as to the pathology of the carrier condition; and no other interpretation which has been put forward is, I think, compatible with all the facts. But I shall have occasion to refer to the discrepancies and difficulties in these other interpretations later in another connexion.

At present there is little to show what percentage of the persons who acquire infection with *E. histolytica* will become healthy carriers, and what percentage will suffer from amoebic dysentery or other diseases. The only figures are those furnished by the experiments of Walker

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* I have seen—in the cat's intestine—ulceration which is only recognizable with certainty in sections examined under the microscope.

† I cite this case because it is one in which I was able, through the kindness of Capt. Armitage, to take a personal interest.

(*vide* Walker and Sellards, 1913). Of 18 men who were experimentally infected with *E. histolytica*, only 4 (22·2 per cent.) developed symptoms of amoebic dysentery—the rest (14, or 77·8 per cent.) becoming contact carriers. Some of them were under observation for over two years, and never showed any signs of dysentery or other amoebic disorders.

It should be noted that 2 of the 4 subjects who developed dysentery had very mild attacks, which might have been overlooked if they had not been under close observation. Moreover, Wenyon and O'Connor (1917) found 106 carriers among 1979 healthy men examined in Egypt; and of these 106 infected individuals they say "only 16 gave any history of dysentery, and it is certain that the latter figure is too high, for in no case can we be certain of the type of dysentery from which the case suffered." Taking these points into consideration, and allowing for the error due to the small number of cases studied, I think too much importance should not be attached to the exact percentages recorded by Walker. It is extremely difficult to obtain reliable information on this subject; but from my own experience I am persuaded that Walker's percentage is too high. I do not believe that more than 10 per cent. of persons who become infected with *E. histolytica* ever suffer to any appreciable extent from their infections; and I think it very probable that even this is much too high an estimate.

Infections with *E. histolytica* appear to be remarkably persistent; and there is good reason to believe that, when an individual once acquires an infection, it will usually—unless he is subjected to specific treatment—persist for the rest of his life.* Cases are known in which infection has lasted for at least 16 years, and probably for much longer periods (Dobell and Stevenson, 1918). Infected individuals may remain healthy, or may show continuous or intermittent symptoms of intestinal derangement, such as diarrhoea or dysentery. A man may be a comparatively healthy carrier for months or even years, and then suffer from an acute attack of dysentery: or he may at any time contract a liver abscess or secondary infection of some other organ from the primary focus in his intestine. But the factors which determine exacerbations, or the spontaneous abatement of symptoms, or the origination of secondary infections of the various organs, are still too obscure for discussion of them to be profitable at present.

Multiplication.—The only process of reproduction which I have ever observed in *E. histolytica* is equal binary fission. Dividing organisms are excessively rare in the stools of human beings, even when suffering from dysentery and passing large numbers of amoebae. In my experience they are so rare, indeed, that they may practically be said to be absent. This is not surprising, when it is remembered that the organisms live—and therefore probably multiply—in the tissues. Those which are washed out from the intestine invariably die, and as a rule rapidly. It thus seems clear that the reproductive stages must be sought in the ulcers in the wall of the bowel. As this is impossible in the case of human infections, I had recourse to the cat: for in this animal the amoebae multiply with great rapidity, and it is therefore comparatively easy to obtain ulcers in any desired stage of development and amoebae

* Cf. especially Walker and Sellards (1913), Wenyon and O'Connor (1917), Dobell and Stevenson (1918).

in unlimited numbers.* After various trials, I found that dividing organisms could only be studied satisfactorily in sections. The infected kitten must be killed, and not allowed to die; and the ulcers in its intestine must be fixed immediately after it has been killed. The least delay in removing the tissues causes the disappearance of dividing organisms or the appearance of abnormal division forms. Scraping the amoebae out of the freshly excised ulcers also gave me unsatisfactory results as a rule. The following account is based, therefore, upon a study of the dividing amoebae found in the ulcers of kittens experimentally infected with *E. histolytica*, and studied in serial sections of material fixed and stained by various reliable methods. I have now studied a large amount of material from kittens—obtained during the investigations undertaken with Dr. H. H. Dale in 1916†—and I have been able to study the process of division in considerable detail. It may be noted, however, that it is by no means so easy to obtain all the stages of division as one might suppose. One may section many ulcers without being rewarded by finding a single stage.

The process of division in *E. histolytica* is closely similar to that of *E. ranarum*, and the division of the nucleus is almost identical with that which I have previously described in the cysts of the latter species (Dobell, 1909). I have illustrated the chief stages in *E. histolytica* in figs. 43-54, Pl. III, which almost describe themselves.

The first recognizable stage in division (fig. 43) shows an increase in the volume of the nucleus, a fragmentation of the karyosome, and apparently a migration of chromatin from the nuclear membrane towards the centre. Such stages are easily recognized, and are connected by numerous transitional stages with organisms showing ordinary resting nuclei (*e.g.*, fig. 1, Pl. I). The chromatin granules gradually become more numerous, the outline of the nucleus becomes oval, and achromatic threads appear within it—usually more or less longitudinally disposed (fig. 44). I have been unable to make out any definite arrangement of the chromatin granules at these stages: and although the nuclear membrane stains readily now, and at all subsequent stages, it seems to be no longer studded with chromatin granules on its inner surface—as in the resting nucleus. The appearances suggest that these granules have passed, for the most part, towards the centre, where they form part of the mass of granules now seen (fig. 44).

The nucleus next becomes fusiform, at first having the shape of a small stumpy spindle (fig. 45), and later of a long slender one (fig. 46) which ultimately stretches right across the organism (fig. 47). I have studied all these stages with great care, but they are very puzzling. At first sight the spindles suggest mitotic figures, with chromosomes and achromatic spindle-fibres. More careful investigation has always convinced me, however, that the irregular masses and threads within the nuclei cannot be resolved into the definite chromosomes and other structures of a true mitotic figure. Fig. 45, which is drawn from a rather small individual at an early stage, will illustrate my meaning. The granules and threads within this nucleus strongly suggest a mitosis.

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* Contrary to Swellengrebel and Schiess (1917), and some other observers, I find that the amoebae in the cat are morphologically identical with those in man. Cf. Dale and Dobell (1917).

† *Vide* Dale and Dobell (1917).

I cannot count the "chromosomes," however, and I cannot resolve the structures here seen into a typical mitotic figure. Similar stages have similar appearances, but the numbers of "chromosomes" and fibres appear to be variable. In later stages (fig. 46) the figures are even worse to study, and show a most confusing arrangement—or apparent lack of arrangement—of stainable threads, masses, and granules. There are, however, usually several definite achromatic fibres which pass from one end of the spindle to the other. They can sometimes be seen with great clearness, and even counted (fig. 47), but their number seems to be inconstant and their arrangement often varies. Not infrequently—as in fig. 47—they are crossed or twisted towards the middle of the spindle. (This figure is drawn from an iron-haematoxylin specimen, very strongly differentiated.)

The greatly elongated spindle now constricts in the middle, its internal structure undergoing no obvious change (fig. 48). The constriction becomes more marked (fig. 49), and the two ends now pull apart (fig. 50), but still remain connected for some time by a thread. This then snaps, and the resulting daughter nuclei become rounded and vesicular (fig. 51). At this stage they begin to show a definite arrangement of the chromatin once more—some of the granules passing to the periphery, and one of them, slightly larger or more conspicuous than the others, often being recognizable as the karyosome of the new nucleus. During the earlier stages of division the spindle is typically sharply pointed; and the points frequently persist until quite a late stage (fig. 50). Sometimes the ends become rounded earlier, however, before the daughter nuclei are fully formed (fig. 49). The specimens figured have been selected to show these slight variations.

The end stages in fission are very simple. The two daughter nuclei undergo reconstruction, into the form of the resting nucleus, by gradual rearrangement of the chromatin granules on the nuclear membrane and differentiation of the karyosome in the centre. The whole organism becomes elongated, and the nuclei pass to its ends (fig. 52). A constriction then appears in the middle of the animal (fig. 53) and gradually deepens until complete constriction into two is effected (fig. 54). Remnants of the thread which originally connected the two daughter nuclei often persist for a considerable time, as little knobs or outgrowths on the daughter nuclei (cf. figs. 52, 53). Late stages in fission, it may be added, are extremely difficult to obtain. This is because the dividing amoebae are usually tightly packed together in the bases of the ulcers: and when division of the cytoplasm takes place, they still remain closely crowded. It is thus very difficult, in sections, to be certain whether two small amoebae in close apposition are dividing or divided forms, or merely two small unrelated organisms accidentally in contact. Figs. 53 and 54 were drawn from specimens lying in the mucus on the surface of an ulcer, where they were somewhat isolated. They were found with difficulty, as dividing organisms are rare in such situations.

The foregoing account of the nuclear division is based, as already noted, on a study of a large number of dividing organisms. It is somewhat unsatisfactory because the nuclear divisions are so peculiar that they are difficult to describe in ordinary terms. Whether chromosomes are present I am still unable to decide, as the nuclear figures are very difficult to interpret. The illustrations are drawn as carefully as possible, so that the reader will, I hope, be able to put his own construction on the actual

events depicted. For my own part, I do not consider the nuclear division of *E. histolytica* to be a regular mitosis. On the other hand, I cannot call it an amitosis. It seems rather to belong to an intermediate category, like the nuclear division of *E. ranarum*.

A word may be added regarding the possible presence of a centriole. It will be noted that I have not so far described a centriole—for the reason that I have not found one. In the early stages—*e.g.*, that of fig. 43—I have never succeeded, by any method of staining, in detecting a centriole. In later stages, such as figs. 44 and 45, "centrioles" can, no doubt, be discovered by the ingenious. Their selection is not difficult, to the willing, from the number of granules and threads at one's disposal. Of the existence of a real centriole, however, I am unable to convince myself. I have made a special study of all the spindle figures such as those shown in figs. 45-47: because it seemed to me that if centrioles were present they should here be discoverable at the poles. But I have not found them. Occasionally—as in fig. 47—a granule may be visible at one pole, or more rarely at both. Sometimes, also, threads appear to join these granules, forming a "centrodesmose." But I attach little importance to these, as they may be found in different positions in different nuclei; and if they represent centrioles or their derivatives, then these structures must display a degree of mutability and variation in behaviour which makes their relation to nuclear division open to the gravest doubts. In short, I have found no structures which I can regard, with any confidence, as centrioles or centrosomes in *E. histolytica;* and in this respect my findings agree completely with those previously recorded for *E. ranarum* (Dobell, 1909, 1914).

The division of *E. histolytica* has been partially described by several workers, but nobody appears to have studied all the stages previously. Schaudinn (1903) stated that the organism divides into two, and that the nuclear division is an amitosis; but he did not describe the process. Most of the published figures depict organisms in various stages of degeneration—not in division. For example, the "dividing" forms of Werner (1908), and Hartmann's (1908, 1912 *a*) "prophases" with "dividing centrioles," are probably not division stages at all. It is significant that Hartmann never found the later stages of division. Brumpt (1913, p. 24, fig. 9) has figured some division stages in amoebae from the cat, but these also are incomplete and partly abnormal. The nuclear division is not completed in the way suggested by his figures. Job and Hirtzmann (1916) say that the nucleus divides by "amitosis," but give no description or figures. It seems probable that they never observed real dividing forms. Mathis and Mercier (1916 *b*) have given a brief description of division in *E. histolytica*, but it is incomplete and faulty, as they apparently saw only a very few stages in human stools. I cannot confirm their statement that a centriole is present; but until they publish a fuller account, with figures, of the stages which they actually saw—apart from their interpretations—it is impossible to discuss satisfactorily the discrepancies in their description. It should be noted, however, that they deny that the "*histolytica* forms" of *E. histolytica*—the large tissue-inhabiting forms, which contain red corpuscles—undergo division at all. These, of course, are the very forms which do divide, and which constitute the bulk of the species. They are the forms whose divisions I have just described in detail.

The earliest observations* on the division of *E. histolytica* appear to have been made by Harris (1894), who saw division occur in the living organisms. He saw several specimens divide into two, but gave very imperfect figures. A division into three which he once saw was doubtless a pathological process of fragmentation. According to Harris's account, the daughter individuals remain attached for a time by a slender connecting thread of cytoplasm. I have never been able —in spite of many attempts—to observe division in living specimens of *E. histolytica*. Harris says he sought for it in vain for three years. He finally saw it in the stools of only one patient suffering from acute amoebic dysentery. At his first observation he saw one amoeba divide into three; at a second he saw "several amoebae" divide into two; and at a third, "division was again observed" (into two?). No cytological details were made out.

It should be added here that the binucleate specimens of *E. histolytica*, which sometimes occur in the stools, and which many observers have seen and noted, are not normal division stages. They are, I believe, forms which were undergoing division at the time when they were passed out of the body, and in which nuclear division has then been completed without fission of the cytoplasm following. As is well known, the sudden cooling of cells or organisms during division usually causes an arrest or regression of the process; and the appearance of binucleate individuals of *E. histolytica* in stools is probably to be similarly explained as a result of the sudden change of temperature experienced on leaving the host. These binucleate forms I have sometimes watched for a considerable time, but they have never completed their division.

Cutler (1919) has recently published an account of the division of *E. histolytica* which is largely incorrect, and is based upon a study of degenerate individuals in human stools—so far as I can judge. He does not appear to have seen most of the stages which I have here described. He believes that there is a peculiar "chromatin extrusion" during the division of the nucleus; but from his account it seems to me probable that this is a degenerative phenomenon. Certainly no such process occurs in the organisms which I have studied—nor in the nuclear division of any other protozoon with which I am acquainted.

A process of multiple fission or schizogony has been described in "*Amoeba dysenteriae*" (=*E. histolytica*) by Job and Hirtzmann (1916). What their "morulae" supposed to be so formed really were I am unable to decide from their description and figures. They certainly were not stages in the normal development of *E. histolytica*, in any case, as Mathis and Mercier (1916 *b*) have already pointed out. The whole account is highly suspicious and unconvincing. More recently the same authors (Job and Hirtzmann, 1919) have reaffirmed their belief in the existence of a schizogony, but without adducing any evidence.

Encystation.—Before the amoeboid forms of *E. histolytica* encyst they undergo a reduction in size, with the formation of precystic individuals

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* Kruse and Pasquale (1894) figured what they thought might be dividing amoebae, but they were unable to observe division. Their figure appears to depict either two separate amoebae in contact, or else a couple of large cells from the stool. Doflein (1901) suggested that these forms might be stages in "conjugation"—a suggestion which seems quite unjustified at the present day.

which differ in several respects from the tissue-inhabiting forms. The reduction of size is doubtless effected by division, though the process has not yet been observed. The small precystic amoebae were originally described by Elmassian (1909) as a distinct species—*E. minuta*—and are sometimes still called "minuta" forms in consequence. Their correct interpretation* we owe to Walker (1911, 1913), though I had previously observed and described an exactly comparable phenomenon in the development of *E. ranarum* (Dobell, 1908, 1909).

The precystic forms of *E. histolytica* are intermediate in size between the large tissue-forms and the cysts—the smallest of them being, of course, of the same size as the cysts which they form. There is thus a considerable range of variation in their dimensions. Those races which produce cysts of small size have correspondingly small precystic forms, whilst the strains with large cysts have precystic stages of appropriately larger sizes. In all strains, however, the precystic amoebae have the same general structure. (See figs. 77-80, Pl. IV.) They are entirely free from all food inclusions—which are eliminated by digestion or excretion before encystation—and are, when alive, sluggish or sessile. Their nuclei have the same general structure as those of the large forms, but the peripheral chromatin is often in a slightly thicker layer, and the karyosome is often slightly larger in proportion, and sometimes—though not often—slightly displaced from the centre. There is also, sometimes, a small amount of chromatin in the zone between the karyosome and the nuclear membrane (cf. fig. 2, Pl. I). In all these respects the precystic amoebae approximate in structure to *E. coli*, from the precystic forms of which those of *E. histolytica* are often difficult and sometimes impossible to distinguish. (Cf. figs. 2 and 13, Pl. I.)

The precystic amoeba comes to rest, becomes rounded, and secretes a cyst wall, thus becoming completely encysted. Encystation occurs only in the bowel—the precystic amoebae which are passed out with the stools being apparently unable to complete their development outside the body. In my experience they invariably die without encysting. The "encystation" of *E. histolytica* outside the body has recently been described by Yoshida (1918); but his figures unmistakably depict a variety of abnormal and degenerate amoebae undergoing fragmentation and other pathological changes. His experiments appear to show, however, that *E. histolytica* may, under certain conditions, survive—in a more or less degenerate state—outside the body for a considerable time (up to 72 hours).

Cysts.—The normal development of the cysts of *E. histolytica* is as follows. The encysted or encysting organism forms, in its cytoplasm, blocks or masses of a highly refractile substance which gives all the reactions of chromatin. I call these *chromatoid bodies*,† but they have been given various other names (chromidia, crystalloids, inclusions, etc.). Whether they are formed from the chromatin of the nucleus, or are secreted in the cytoplasm, is still uncertain. Their staining reactions

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* Confirmed by Darling, Wenyon, and many other workers soon afterwards.

† I have used this term for some years, as it seems to me the most suitable. (Cf. Dobell and Jepps, 1917.) It is unnecessary to enter into the prolonged argument which has recently taken place between Chatton (1917, 1918*a*) and Mathis and Mercier (1917*e*, 1917*f*) on this subject. It merely shows the lacunae in our knowledge, and the different interpretations which different observers can put upon the same appearances.

prove nothing* in this respect, and all that can be said with certainty is that they appear in the cytoplasm, where they increase in size. When fully developed they are most commonly in the form of a few fairly large blocks or bars with rounded ends (cf. figs. 3-5, Pl. I).

At the time when the chromatoid bodies make their appearance, a vacuole also forms in the cytoplasm (fig. 3). It is of variable size, and sometimes two or even three are formed instead of one. The vacuole is stained brown in iodine solution, but not so deeply as the corresponding structure in the cysts of the other intestinal amoebae. In iodine solution its edge is not as a rule sharply defined. The brown-staining substance in the vacuole is glycogen; for it not only gives the iodine reaction just noted, but also shows the characteristic solubilities of this substance, and can be stained by Best's specific carmine method† (fig. 6, Pl. I).

The cyst when first formed is uninucleate (fig. 3, Pl. I). Its nucleus typically measures about one third of the diameter of the whole cyst, or slightly less. The nucleus later divides into two (fig. 4), and each of the daughter nuclei then again divides, so that four nuclei are finally formed (fig. 5). In this quadrinucleate stage the diameter of each nucleus is approximately one sixth of that of the entire cyst, or about half that of the nucleus in the uninucleate cyst. The nuclei of the binucleate cyst are intermediate in size. (Cf. figs. 3, 4, and 5, Pl. I, and figs. 72-76, Pl. IV.) The nuclear divisions within the cyst appear to be exactly like those in the free amoebae, except for the progressive reduction in size. They are also almost exactly like the nuclear divisions in the cysts of *E. ranarum*, which I have elsewhere figured in detail (Dobell, 1909). At all stages in development the resting nuclei in the cysts of *E. histolytica* have a structure exactly like that seen in the free forms. It is therefore unnecessary to describe it again. One noteworthy feature may be mentioned, however. In 2-nucleate or 4-nucleate cysts, the nuclei often show a characteristic condensation of chromatin at one pole, so that the "ring" of chromatin appears slightly thickened at one side (see fig. 5). This also I have described previously in *E. ranarum*.

When the cyst has reached the 4-nucleate condition, no further nuclear divisions occur. The glycogen vacuole, however, generally disappears, so that the mature cyst comes to stain uniformly pale brown throughout in iodine solution. If the mature living cysts are kept under observation, they can also be seen to lose their chromatoid bodies‡

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* James (1914) apparently believes that the chromatoid bodies must be cytoplasmic in origin because they can be coloured blue by Mann's method. But they can also be coloured red by this stain (cf. figs. 3-5, Pl. I), and it is even possible to stain some blue and some red in the same cyst. I have excellent specimens showing them blue, red, and all intermediate shades of purple in the same preparation.

† As first shown by Kuenen and Swellengrebel (1913).

‡ Malins Smith (1918) has recently stated that "the supposition of some writers (Hartmann, 1912, James, 1914) that chromatoid bodies tend to disappear as the cyst becomes mature is not borne out by the facts." That the mature cysts, with 4 nuclei, lose their chromatoids when kept outside the body, is, however, a fact. I first showed this to occur in *E. ranarum*, and have since confirmed it repeatedly in *E. histolytica*. It is also confirmed by Chatton (1917 *b*). If Smith means that the cysts do not lose their chromatoids in developing from the uninucleate to the quadrinucleate stage, then he is probably correct. Nobody has yet maintained that this occurs, however, so far as I am aware. Smith appears to have misunderstood what Hartmann wrote—as reference to the passage which he quotes from him seems to indicate.

gradually in the course of a few days. Cysts which, when freshly passed, show chromatoid bodies in the majority, at the end of about a week show no chromatoids in the majority. The chromatoids can also be seen to grow smaller and disappear in individual cysts examined from day to day. It thus appears probable that both the glycogen and the chromatoid bodies represent reserve materials of some sort—the former being used up while the cyst is developing, and the latter being absorbed in the mature cyst whilst it is waiting to be ingested by a new host. As a rule the cysts contain no other inclusions than those already noted.

In size the cysts of *E. histolytica* are subject to great variation. Their diameters range from about 5 μ as a minimum in the races producing small cysts, up to about 20 μ as a maximum in races forming large cysts. Each race produces cysts of a constant average size, though showing the usual degree of variation round the mean. The figures on Plates I and IV will give a good idea of the difference in size observable in different races. The cysts shown in figs. 72-74, Pl. IV belong to a race with cysts having an average diameter of 6·6 μ: those shown in figs. 3-5, Pl. I are from a race with cysts of an average diameter of 13·5 μ; and those in figs. 75, 76, Pl. IV are from a race forming cysts with an average diameter of 15 μ.* Except for their size, there are no constant morphological differences between the cysts belonging to different strains.

In form, the cysts of *E. histolytica* are typically spherical or ovoid, though they are not as a rule perfectly symmetrical; but their asymmetry is often extremely slight.

The cyst wall, as in other *Entamoebae*, is colourless and perfectly smooth. It is formed of a single layer, so far as I can determine, and measures about 0·5 μ in thickness† in cysts of medium size (ca. 12 μ); but it is slightly thicker in larger and correspondingly thinner in smaller cysts.

The specific gravity of the cysts of *E. histolytica* is about 1065, according to Ujihara (1914), who also found that the cyst wall is almost insoluble in gastric juice, readily soluble in trypsin, slightly soluble in bile, but resistant to lipoid solvents, such as sodium taurocholate and saponin. Its exact chemical composition is still uncertain.

The cysts of *E. histolytica* will survive for several weeks outside the body of man, if they are kept moist and cool. They will live in damp faeces or in water without showing any conspicuous change save the loss of their chromatoid bodies. As a rule, if the cysts are kept under observation, it will be found that some of them remain alive much longer than the others. In water or faeces some will usually be found dead at the end of a week, many more after the lapse of a fortnight, and after this period only isolated survivors will be discoverable. The longest time of survival which I have observed is five weeks (cysts kept in water), but as a rule they will not live so long. Desiccation kills them immediately, and they degenerate much more rapidly at a high

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* These figures are the average sizes of the living cysts. In stained specimens the cysts show an apparent reduction in diameter of about 10 per cent., as I have shown elsewhere in a joint paper (Dobell and Jepps, 1918).

† Some observers (Kuenen and Swellengrebel (1913), Woodcock and Penfold (1916), Brug (1917 *b*), etc.), state that the cyst wall has only a "single contour" when seen under the microscope. It has only one layer, it is true; but in optical section both the inner and the outer surface can be seen and the distance between them measured. The "single contour" appears to be due to an incorrect adjustment of the microscope.

than at a low temperature. At body temperature they generally die within a few days at most. Degeneration of the cysts is readily recognizable. The nuclei first become unnaturally distinct in the fresh cysts —owing to the coagulation which occurs on the death of the protoplasm—and then break up. As the cysts die they also become permeable to aqueous solutions of various stains (eosin, etc.). The cytoplasm becomes vacuolated, and finally disintegrates.

Cysts are passed in the faeces in the uninucleate, binucleate, or quadrinucleate stage. Those containing less than 4 nuclei never develop to maturity outside the body, and usually die much sooner than the mature cysts. Even cysts with dividing nuclei do not complete their nuclear divisions. Spindle-figures and other stages arrested in division can be seen to remain unchanged within the cysts until degeneration takes place.

Numerous observations on the vitality of the cysts have already been recorded. (Cf. Kuenen and Swellengrebel (1913), Wenyon and O'Connor (1917), etc.) My observations are in general agreement with those of others, and with my earlier observations on the cysts of *E. ranarum*.

Several common variations in the contents and form of the cysts of *E. histolytica* must be noted. These concern chiefly the chromatoid bodies and the shape of the cyst as a whole. As regards the former, it may be noted first that the chromatoids are sometimes formed in the precystic amoebae before the cyst wall is secreted (see fig. 82, Pl. V). This is not very uncommon. Secondly, the chromatoid bodies show considerable variation in form. Although typically few and in the form of thick short rods, they may be very numerous (fig. 70, Pl. IV) and of many different shapes—long thin rods, filaments, round or irregular masses, granules, etc. (Cf. figs.3-5, 70, 72-76.) Sometimes they are completely absent (fig. 71) in cysts at all stages of development.

Malins Smith (1918) has recently attempted to determine the frequency with which chromatoid bodies occur in the cysts of *E. histolytica*. He found that they were present in 27 per cent., absent in 65 per cent., and doubtful in 8 per cent. of 1162 cysts which he examined. Unfortunately he did not take into account the fact that the chromatoid bodies disappear gradually from the cysts after they have left the body, and he does not state how long his cysts had been kept before he examined them. Moreover, his figures are based upon the study of living cysts in saline, or those examined in iodine solution* —not upon stained specimens. It is certain that he would have found a much higher percentage containing chromatoid bodies had he examined only freshly-passed cysts in carefully stained preparations. His figures can thus hardly be accepted as a correct estimate of the frequency with which chromatoid bodies occur normally in the development of the cysts of *E. histolytica*.

Although the cysts of *E. histolytica* are typically fairly symmetrical, they may have the most bizarre shapes. Slight irregularities in outline are commonly seen, and occasionally an infected person will pass a stool in which almost every cyst is irregular in shape. Abnormalities in

* It should be noted that small chromatoid bodies—and sometimes even large ones —are difficult to distinguish in cysts mounted in iodine solution.

shape may take almost any form—bulgings, constrictions, and various distortions—so that they are difficult to describe. In general they are similar to the irregularities so frequently encountered in the cysts of *I. bütschlii* (cf. text-fig. 2, p. 115). I have seen pear-shaped, rod-shaped, hourglass-shaped, L-shaped, and variously shaped cysts of other forms, including many which are rhomboidal or triangular in outline. I have twice seen "twin" cysts, formed of two complete cysts united at their point of contact, and with their contents completely continuous.

Nuclear abnormalities are not uncommon. Cysts containing three nuclei—one large and two small—or nuclei of different sizes and abnormal shape or structure may often be found.

Do the Cysts of E. histolytica *ever contain more than Four Nuclei?*—As a rule the cysts of *E. histolytica* contain, when mature, four nuclei. At this stage development ceases. Some workers, however, believe that occasionally all the nuclei again divide, so that a cyst containing eight nuclei is finally formed. No worker claims to have observed more than eight nuclei in a cyst of this species: but as this number is that characteristic of *E. coli*, which so frequently accompanies *E. histolytica*, it is of some importance to ascertain whether the latter species ever produces similar cysts.

Kuenen and Swellengrebel (1913) claimed to have found cysts of *E. histolytica* containing 5, 6, and 8 nuclei, and this was reasserted later by Swellengrebel and Schiess (1917). The evidence adduced is, however, most unconvincing: and I think it more than probable that these authors mistook small cysts of *E. coli*, occurring in a mixed infection, for those of *E. histolytica*. Kuenen and Swellengrebel (1913) state that *E. coli* occurs "only seldom" in Deli, where they worked. This can hardly be correct, for *E. coli* is so common everywhere else; but it implies—to my mind—that they overlooked or misinterpreted many *E. coli* infections. Again, they cite a case in which the 8-nucleate cysts of *E. histolytica* were found in company with "*minuta*" amoebae of this species containing bacteria and starch grains. Now the pre-cystic amoebae of *E. histolytica* do not eat either of these things, but small *E. coli* amoebae, which are easily mistaken for them, frequently do. I have no doubt that their patient was really infected with *E. coli*, on this evidence alone. Further, these authors give 16 μ as the minimum diameter of the cysts of *E. coli*, and they evidently reckoned all cysts with a smaller diameter as belonging to *E. histolytica*—a very common mistake: yet typical 8-nucleate cysts, undoubtedly belonging to *E. coli*, may be found with considerably smaller diameters—at least down to 11 μ. Another point which requires notice is their statement that the 8-nucleate cysts of *E. histolytica* which they found "must" have belonged to this species because the cyst walls showed only a "single contour." This, as already noted,* is an incorrect statement about either species, and rests upon an error in observation. It seems to me certain, therefore, that Kuenen and Swellengrebel were mistaken, and that their 8-nucleate "cysts of *E. histolytica*" were really in every case cysts of *E. coli*.†

Brug (1917 *b*) also believes that he has seen at least one cyst of

* See p. 47, footnote.

† The structure of the nuclei within these cysts is not described, though this is the chief character by which a correct specific determination can be made.

E. histolytica containing 8 nuclei. His evidence is fairly strong, but is not completely convincing, as Smith (1918) has already pointed out; for he has not excluded the possibility of a mixed infection. He does not say how often he examined the stools of his patient: he merely states that they were "often" examined, and that *E. coli* cysts were "always absolutely absent"—a very rash statement to make about any stool. He does not sufficiently appreciate, apparently, the fact that the stools of a person infected with *E. coli* may be examined on scores of occasions without the infection being detected.* The 8-nucleate cyst which he found measured 12 μ in diameter: but undoubted cysts of *E. coli* also occur of this size. The cytological characters of the nuclei are not given, and without knowing these it is impossible to be absolutely certain of the species to which his cyst belonged.†

Mathis and Mercier (1917 *b*) deny that *E. histolytica* ever forms cysts with more than four nuclei. Most other workers who have had a very large experience are also extremely doubtful regarding their occurrence: and there can be no doubt whatever that, if they do occur, they are excessively rare.‡ I have examined some hundreds of thousands of cysts of *E. histolytica*, and I have seen but three which I believe to have been 8-nucleate cysts of this species. That this interpretation is correct, I am unable to prove conclusively, on account of the great difficulty—amounting in practice almost to an impossibility—of excluding a concomitant infection with *E. coli*. I have not yet succeeded in finding an 8-nucleate cyst of *E. histolytica* in my stained preparations;§ and the cytological details, especially as regards the finer nuclear structure, cannot be made out with complete certainty in iodine solution—the medium in which my cysts were examined.

It seems almost certain, however, although the evidence so far is inconclusive, that 8-nucleate cysts of *E. histolytica* must occur. All the other cyst-forming amoebae of man occasionally form supernucleate cysts, containing a double number of nuclei. Cysts of *E. coli* with 16 nuclei are not excessively rare; 8-nucleate cysts of *E. nana* also occur: and binucleate cysts of *I. bütschlii* may, on very rare occasions, be found. Of the related amoebae in other animals, *E. ranarum*—whose 4-nucleate cysts are very closely similar to those of *E. histolytica*—very rarely forms 8-nucleate cysts, according to Epstein and Ilovaiski (1914). I have never seen cysts with more than 4 nuclei in this species (cf. Dobell, 1909), although I have now studied a considerable number. *E. aulastomi*, another entamoeba with 4-nucleate cysts very like those of *E. histolytica* and *E. ranarum*, also very rarely forms 8-nucleate cysts: for Nöller (1912), who discovered this species, once found a single cyst with 8-nuclei. In all these organisms there is little chance of the cysts

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* For some important data bearing on this subject see my earlier work (1917), and also compare Smith (1918).

† Brug does not use the "single contour" of the cyst wall as a specific character, but for the singular reason that only a "single contour" is observable in the cysts of both *E. coli* and *E. histolytica!*

‡ *Vide* Wenyon and O'Connor (1917), Dobell and Jepps (1917).

§ I have, however, seen a stained cyst in a preparation made from one of my cases by Miss Jepps: and from the cytological characters of this cyst, and the history of the patient from whom it came—a patient whom I examined many times—I have little doubt that it is an 8-nucleate cyst of *E. histolytica*, and not of *E. coli*.

having been incorrectly identified, or of their having been confused with those of other species. The supernucleate cysts of *E. coli*, *E. nana*, and *I. bütschlii* are not to be confused with the cysts of any other amoebae in the same situation; and neither the frog nor the leech is infected—so far as is known—with a species of *Entamoeba* normally forming 8-nucleate cysts.

I think it certain, therefore, that *E. histolytica* sometimes forms supernucleate cysts containing 8 nuclei: but this happens so very rarely that, for all practical purposes, such as diagnosis, their occurrence can be ignored.

Races distinguishable by the Size of their Cysts.—As I have already discussed this subject fully elsewhere in a joint paper (Dobell and Jepps, 1918), I shall here merely recapitulate the chief conclusions there drawn, and make one or two additions.

The fact that *E. histolytica* is a species which is composed of different races distinguishable by the size of their cysts, was first definitely stated by Wenyon and O'Connor (1917). I reached the same conclusion independently, and have given the evidence for it in two previous joint papers (Dobell and Jepps, 1917, 1918). In the second of these we were able, I think, to place the matter beyond all reasonable doubt. It was there shown, by the analysis of careful measurements of large numbers of cysts from different infections, that "*E. histolytica* is a collective species. It comprises a number of distinct races, strains, or pure lines, distinguishable from one another by the size of the cysts which they produce. How many such distinct races exist is still undetermined, but we have demonstrated the existence of at least five. There is no evidence that the different races differ in their geographical distribution, or in any character save size. These races remain constant in character within a given host; and the dimensions of the cysts are not determined by the action of the host upon the parasite, since two different races may coexist side by side in the same host."

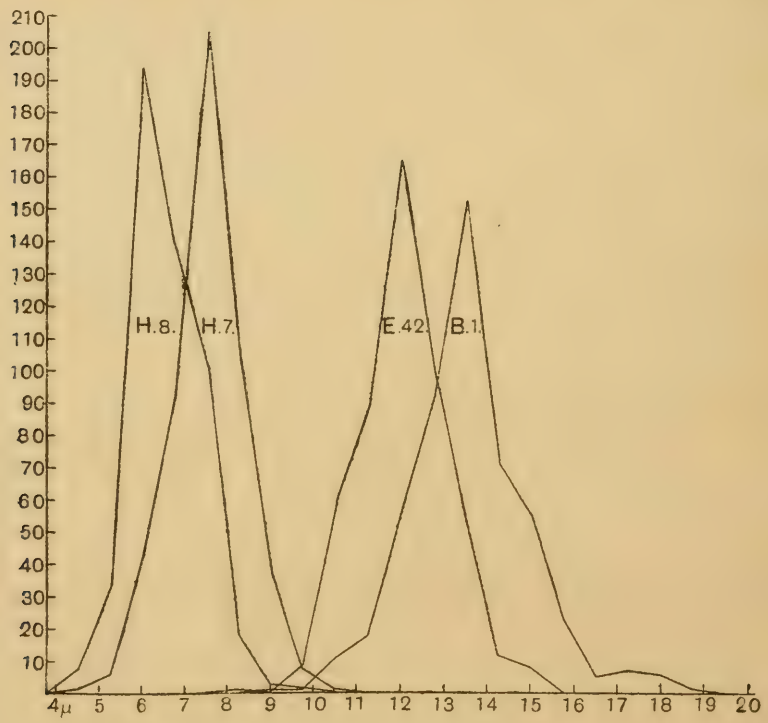
The five races of *E. histolytica* which we specially studied had cysts with mean diameters* of 6·6 μ, 8·3 μ, 11·6 μ, 13·3 μ, and 15 μ. Text-fig. 1, here reproduced (p. 52) from our paper, will show at a glance the striking differences in the dimensions of the cysts belonging to four of these races (Cases H.8, H.7, E.42, B.1). The curves were plotted from measurements of 500 cysts from each race, and were superposed subsequently. The ordinates show the number of cysts measured, the abscissae their diameters in microns.†

Races of *E. histolytica* which produce cysts of the smaller sizes (usually 7 μ—9 μ in diameter) were long overlooked. They appear to have been first recorded by Prowazek (1912*a*), who found them in Samoa, and regarded them as belonging to a distinct species which he named "*Entamoeba hartmanni*." In the same year they were also found by Aragão (1912) in Brazil, and combined by him with *E. coli* to form

* These figures were calculated for living cysts—the measurements having been made upon fixed and stained specimens. The differences in size between living cysts and those fixed, stained, and mounted in balsam, were investigated in detail, and the factors determining these differences ascertained. *Vide* Dobell and Jepps (1918).

† The diameters here shown are those of stained cysts mounted in balsam, in which medium the apparent diameter of a cyst is about 10 per cent. less than its true diameter when measured alive in saline solution.

another "new species" called "*E. brasiliensis.*" They were seen later by Ujihara (1914) in Formosa, and correctly referred to *E. histolytica.* Ujihara* apparently studied five different strains of *E. histolytica,* with cysts of the following mean diameters approximately: 6·8 μ (1 case), 8·5 μ (3 cases), 10·24 μ (3 cases), 11·95 μ (7 cases), 13·6 μ (4 cases) Unfortunately the author does not state how many cysts he measured from each case, nor how he made his measurements. It is impossible to know what exact value to attach to his figures in consequence.



TEXT-FIG. 1.—After Dobell and Jepps (1918).

Nevertheless, there is a striking similarity between four of his races and four of those which I studied with Miss Jepps, as will be apparent from the following table:—

	Ujihara (1914).	*Dobell and Jepps* (1918).
Race 1	6·8 μ	6·6 μ
„ 2	8·5 μ	8·3 μ
„ 3	10·24 μ	—
„ 4	11·95 μ	11·6 μ
„ 5	13·6 μ	13·3 μ
„ 6	—	15·0 μ

* In my earlier papers (Dobell and Jepps, 1917, 1918) I unfortunately overlooked the observations of this Japanese worker—partly, I think, because he himself did not call attention to the significance of his findings, which were merely recorded in a table with other data. I did not realize their importance until I recently re-read his paper.

As regards the race called "3" in this table, it should be added that Wenyon and O'Connor (1917) have studied one somewhat similar (their case "Kettlewell"): and I have also seen at least one similar infection, in which the cysts ranged round a mean of about 10 μ.* The race called "6" in the table, is absent from Ujihara's series. It undoubtedly exists, however, as I have studied several cases; and Wenyon and O'Connor (1917) have recorded a case of the same type (case "Healy"), with large-sized cysts reaching 18 μ in diameter. The large cysts of these strains of *E. histolytica* are sometimes attributed to *E. coli*. I know of several actual instances where this mistake has been made in the laboratory.

James (1914) found cysts of *E. histolytica* measuring 7-10 μ in diameter, and referred them to their proper species.† Woodcock and Penfold (1916) again described small-sized cysts of *E. histolytica*, but proposed to call them provisionally "*E. minuta*." This was an unfortunate application of the name originally proposed by Elmassian (1909); and although Woodcock (1917) still adheres to this nomenclature, there is clearly no justification for it.‡

Swellengrebel (1917) has described small cysts of *E. histolytica*, but attributed them to the flagellate *Chilomastix mesnili*. Kuenen and Swellengrebel (1917) in the same year again described them, but regarded them as the cysts of a new species of *Entamoeba*, which they proposed to call *E. tenuis*. It may be noted as a curiosity, however, that Kuenen and Swellengrebel (1913) had previously referred Prowazek's *E. hartmanni*, which was the same thing—*i.e.*, a race of *E. histolytica* producing small cysts—to "*E. tetragena*," which is their name for *E. histolytica*. They have given no reasons for these inconsistent statements. Brug (1917) has also found the small cysts of *E. histolytica*, and has also regarded them as belonging to a new species, for which he proposed the name *E. minutissima*. Later, however, Brug (1918) withdrew this name in favour of that of Kuenen and Swellengrebel. It is thus difficult to understand what the Dutch workers' present views really are about the strains of *E. histolytica* which produce small cysts; and it is possible that they will undergo still further changes when they have studied the publications of others. At present they appear to be unaware of the large amount of work which has been done on this subject by English investigators.

Mathis and Mercier (1917 *g*) are unwilling to believe in the existence of the strains of *E. histolytica* with cysts of different sizes. Their doubts were expressed, however, before the appearance of my full paper on the subject (Dobell and Jepps, 1918). Various objections which they raised have there been answered, though some of their criticisms appear to me irrelevant. It may be noted that they regard all cysts of *E. histolytica* with diameters of less than 10 μ as "abnormalities"—a view which is certainly untenable. It may be noted further, as

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* *Vide* Dobell and Jepps (1918), p. 540.

† Called by him *E. tetragena*. James appears to have regarded the small cysts as abnormal, for he says "probably some circumstance of environment was responsible for their condition" (1914, p. 187). This hypothesis can hardly be maintained now that it has been proved that two strains—a small and a large—may exist in the same host simultaneously (Dobell and Jepps, 1918).

‡ Cf. Dobell and Jepps (1917, 1918).

somewhat curious, that these authors—who deny that *E. histolytica* has races producing cysts of small size—have nevertheless included "*E. hartmanni* Prowazek, 1912" in their list of synonyms of "*E. dysenteriae*," which is the name they give to *E. histolytica* (Mathis and Mercier (1916) p. 644); though Prowazek's "*E. hartmanni*" was actually a strain of *E. histolytica* producing small cysts.

Malins Smith (1918) has recently cast doubts upon the existence of more than two strains of *E. histolytica*. He admits that there is an "ordinary strain" having cysts with an average diameter of 12·6 μ, and a "small strain" with cysts of 7·7 μ. He seems, however, to have reached this conclusion as a result of a fallacious method of investigation. He measured small samples of cysts from 30 different infections (not more than 50 from any individual case), and thus obtained measurements of the diameters of 1000 cysts. Plotting these graphically, he obtained a bimodal curve with modes at 7·1 μ and 12·2 μ. He then says: "There seems to be no doubt from the curve that the cysts of this species divide themselves naturally into two strains, differing only in size, with dimensions as indicated in the curve." But this conclusion is surely unwarranted. If there are strains of *E. histolytica* which differ in the diameters of their cysts—as the figures which Miss Jepps and I have published do, I think, prove conclusively—then Smith's curve does not show how many such strains there are, and what mean diameters they individually possess, but merely the frequency with which strains of different sizes occurred in the sample of 30 infections which he studied. His curve merely shows—on the basis of small samples from each—that strains having the larger-sized cysts were most plentiful in his material, strains with small cysts less numerous, and those with cysts of intermediate and very large size absent. His own figures (his Table I, p. 34), so far as they go, seem to me quite consistent with our results: but his conclusion that there is only evidence of the existence of two strains, with average cyst-sizes of 7·7 μ and 12·6 μ, can hardly be deduced from his curve. He has not even demonstrated the existence of a single strain with either of these diameters as its mean.* No adequate number of individuals was studied from any one strain; and how any legitimate conclusions can be drawn by treating the problem in such a manner I do not understand. One might as well attempt to show that there are no differences in stature among the various European races of man by measuring the heights of a few individuals from each race and then striking an average for the whole lot! It is clearly impossible to show by Smith's method whether racial differences do or do not exist.

It seems to me, therefore, that there is a fundamental fallacy in the method which Smith has adopted; and consequently I do not think it necessary to discuss the details of his work further. Nevertheless, I am glad to note that he admits that the species *E. histolytica* can be divided into at least two different races, distinguishable by the sizes of their cysts; and I have but little doubt that, if he continues his investigations, he will be able to convince himself of the existence of others.

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* His Cases 24, 25, and 26 have an average diameter of 12·6 μ, but only 50, 20, and 37 cysts respectively were measured from each of these. With such small samples the conclusion hardly amounts to a demonstration. I can find no single case recorded in which the cysts had an average diameter of 7·7 μ—a figure which seems to result from taking the mean of at least two distinct strains.

It would be interesting to know whether cysts of different dimensions belong to strains having tissue-inhabiting amoebae of correspondingly different sizes. Up to the present I have not been able to determine this point. An attempt to infect a kitten by means of small-sized cysts —made in conjunction with Dr. H. H. Dale in 1916—was unsuccessful; but the large tissue-forms could doubtless be obtained in this manner.* There is no doubt, of course, that the precystic amoebae of different strains correspond in size to the dimensions of the cysts which they form—strains with large cysts having large precystic amoebae, and strains with small cysts small precystic forms. (Cf. figs. 77-80, Pl. V.) But I have never yet been able to study a case, known to be infected with a strain producing small cysts, in the acute dysenteric stage of the infection: and with the strains producing larger cysts I have not been able to estimate the size of the amoebae with sufficient accuracy to render any comparison possible.

The only relevant figures which I have been able to collect are those published by Ujihara (1914), who has recorded the dimensions of the active amoebae observed in the stools of a number of patients suffering from acute amoebic dysentery, and the dimensions of the cysts found in the faeces of the same cases subsequently. Unfortunately he gives no indication of the number of specimens measured or of his method of measurement—both very important points. His table shows that there is no correlation between the size of the active amoebae and the size of cysts which they produce. Some of the largest amoebae, in fact, were found in cases which subsequently showed the smallest cysts in their faeces. For example, his Case 1 passed, during a period of acute dysentery, amoebae whose diameter is stated to have been 44·63 μ, whilst the cysts from this patient measured 6·83 μ. The corresponding figures from his Case 5 are 30·7 μ and 8·45 μ.

I may add that my friend the late Mr. W. O. Redman King told me that he had studied a mild case of amoebic dysentery which recovered clinically without treatment. In the stools—of which he sent me a specimen—cysts measuring 8-9 μ in diameter were present after the recovery. He informed me that the amoebae passed during the dysenteric attack were not noticeably smaller than those which he had seen in cases infected with strains producing cysts of the larger sizes (about 12 μ); but unfortunately he made no permanent preparations of the amoebae, and did not measure many of those which he saw alive.

At the present moment, therefore, the only certain conclusion which can be drawn is that this question merits further investigation.

The Early Stages of Development. Excystation.—Although the vegetative amoebae and cysts of *E. histolytica* are now fairly well known, the earlier stages of development are still undiscovered. Since Quincke and Roos (1893) first put forward the suggestion, it has been amply proved that man acquires his infection with the parasite by ingesting its cysts;† but

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* Cutler (1919) has stated recently that he infected a cat by means of cysts of a "small type." The animal "developed dysentery and died," and in its intestine he found "a small variety of tissue-invading amoebae." It is stated that sufficient material from this case has not yet been examined, but it is to be hoped that further and more precise information will be forthcoming.

† Numerous workers have, of course, experimentally infected cats and other animals by means of the cysts of *E. histolytica.* See p. 67 *infra.* Quincke and Roos

the stages intervening between ingestion and the establishment of the amoebae in the tissues of the large intestine require further investigation.

Walker (*vide* Walker and Sellards, 1913) fed 20 men on *E. histolytica*, sometimes encysted, and sometimes free—a special technique being employed in the latter case to enable the amoebae to pass through the stomach. He succeeded in infecting 18 out of the 20 men fed by these methods. The infections were established — as determined by the appearance of cysts in the stools—in times varying from 1 to 44 days, the average period being 9 days. The time which elapsed between the infective feed and the attack of dysentery—in four cases which developed dysentery—was 20, 57, 87, and 95 days (average 64·8 days). This last figure is clearly of doubtful value, since it ignores all the cases which did not have dysentery—any of which might subsequently develop amoebic dysentery at any time, and so change the "average incubation period" within indeterminate limits. It may be noted that the "incubation period" in the cat—which, if infected at all, invariably suffers from dysentery, and does not become a carrier — is usually about a fortnight, if the infection is conveyed to it by cysts *per os*. With intrarectal injection of active amoebae it is much shorter as a rule, infection being sometimes established within twenty-four hours.*

Since it is impossible to observe the earliest stages in the development of *E. histolytica* in human beings—their normal host—it appears probable that recourse must be had to animal experiments if further information on this subject is to be obtained. The cat at once suggests itself as a suitable subject for investigation; but unfortunately, it is not so suitable as one might suppose. In the course of work undertaken with Dr. H. H. Dale in 1916, attempts were several times made to infect kittens with cysts administered *per os;* and then, by killing them later, and examining the contents of their intestines, to discover the earlier stages in the development of the amoebae in this animal. Unfortunately all these attempts failed. The only definite results which we obtained showed clearly that cats are not easily infected by this method; and that as a rule the majority of the ingested cysts die in the cat's intestine, though a few will pass through unchanged. We made altogether 17 attempts to infect kittens by these means, using cysts from 7 different human infections, but we succeeded in infecting a kitten only once. It thus seemed to me probable that it would be necessary to make a very large number of experiments, and a great sacrifice of kittens, to obtain conclusions of any value in this way. The attempt was therefore abandoned after a few preliminary failures.

Chatton (1917*b*) appears to have been more successful with this method. He found that the cysts of *E. histolytica*, when swallowed by a cat, passed through its stomach without undergoing any change save the "digestion"† of their chromatoid bodies. In the small intestine,

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made the suggestion on analogy with their results with cats. It should be remembered, however, that Grassi and Calandruccio had previously shown that man acquires infection with *E. coli* by swallowing the cysts of this species.

* With a strain of amoebae which was passed through 106 kittens by rectal injection, the average incubation period was slightly over 2 days (Dale and Dobell, 1917).

† Chatton does not say whether the chromatoids are "digested" in the stomach by the host or the parasite. I take it that actually he merely observed no chromatoids; and that he interprets their absence as an indication that the amoeba has assimilated them

however, the cysts hatched, and each liberated a 4-nucleate amoeba. These amoebae passed down the intestine with the gut-contents, and were found in the large bowel and the evacuations. According to Chatton the newly hatched amoebae actively ingest bacteria, become vacuolated, and their four nuclei become clumped together. Further development was not seen.

Chatton believes his observations to indicate that the cysts of *E. histolytica* normally hatch in the small intestine, liberating undivided 4-nucleate amoebae, which first live upon bacteria and then pass on and establish themselves in the tissues of the large bowel. He interprets the ingestion of bacteria in a phylogenetic sense—as an indication that this species was primitively a commensal, like *E. coli*, before it took to preying upon the tissues. I do not share this view. I think it more probable that his amoebae were degenerate, and invaded by bacteria.* The fact that they were vacuolated supports this interpretation ; for vacuolation is one of the first signs of degeneration in *E. histolytica*. The fact that the nuclei were later found to be agglomerated still further supports it ; for agglomeration of the nuclei is often seen in multinucleate protozoa during degeneration.† It is, of course, impossible to know whether the cats in which these amoebae were found would have become infected if they had not been killed : but Chatton states that none of his controls became infected.‡ It seems probable, therefore, that the cats which were killed would also have remained uninfected. I am thus inclined to think that the cysts, in these experiments, hatched in an abnormal manner, and the amoebae afterwards degenerated and gradually died. The intestine of the cat was sufficiently like that of man—their normal environment for development—to enable them to emerge ; but it was not sufficiently like the human intestine to enable them to develop. The experiments support the view, which is at present the only probable one, that the cysts hatch in the small intestine. But they by no means prove that the cyst normally liberates a 4-nucleate amoeba in man ; or, if it does, that its later development is similar to that observed in the cat. Chatton himself says that "the incomplete development in the cat is due to the cat being not a normal host of the amoeba."

Ujihara (1914) had previously stated that the cyst wall of *E. histolytica* is soluble in trypsin, but almost insoluble in gastric juice. This seemed to indicate the small intestine as the place where the cysts normally hatch : and Penfold, Woodcock, and Drew (1916) have stated that they were able to cause cysts to hatch by placing them in *liquor pancreaticus* (Benger),—in which, however, "only a small proportion excyst." They say : "We have tried pepsin, in an acid medium, bile, and pancreatic extract, either alone, consecutively, or together, as appeared indicated,§ but the only success we have had has been with pancreatic extract used alone." They observed the emergence of a single amoeba from the cyst,

* See p. 62.

† For instance in *Trichosphaerium* and *Actinosphaerium*.

‡ He says : "None of the cats not sacrificed in my experiments has contracted dysentery as a result of ingestion of the cysts."

§ It would be interesting to know the "indications" for acting upon cysts with a mixture of trypsin, in acid solution, and alkaline pancreatic juice. I cannot help thinking that an amoeba—even if quite willing to emerge—would be as much perplexed by being placed in such a mixture as I am in trying to conceive the indications for its use.

through a small aperture. The nuclei of these amoebae were not investigated, however, and they state that "from our living (*sic*) observations, we could not tell whether the amoebae which excysted had always four nuclei." Nevertheless, the authors "are strongly of the opinion that this is the normal method of excystation." The evidence for such strong views is not indicated. The excysted amoebae are stated to have thrown out pseudopodia, but they underwent no further development, and finally died.

Shortly after the publication of these experiments, Dr. A. C. Stevenson and I attempted to repeat them. Although we carefully followed the methods described, we never succeeded in causing the cysts to undergo any development. After a number of failures, we came to the conclusion that *E. histolytica* will not usually excyst in *liquor pancreaticus*. We thought it highly probable that among the cysts which Penfold, Woodcock, and Drew employed, there were some which had only recently been formed; and that the walls of these might occasionally be digested by the pancreatic fluid, and so liberate newly-encysted organisms still possessing some powers of movement. We could find no evidence to prove that the authors had ever caused a mature 4-nucleate cyst to hatch—in spite of their firm conviction. I still think that this is a probable explanation of their observations.

More recently Cutler (1919) has recorded similar experiments. He states that "all the excysted amoebae were uninucleate," and agrees with the explanation just advanced. But he claims to have obtained more satisfactory results by treating the cysts with *liquor pepticus* "for a short time," followed by *liquor pancreaticus*—a method which apparently failed with Penfold, Woodcock, and Drew. By this method, the majority of excysted amoebae are stated to be 4-nucleate, the author having been "able to stain them with methyl green during their emergence." No further development of these excysted amoebae was observed. But Cutler adds that though he has "not seen the intermediate stages there is evidence that these quadrinucleate amoebae ultimately divide to form four small amoebulae." Except, however, for a figure of a small amoeba —whose origin is not indicated—the evidence is withheld.

It will thus be clear, I think, that the early stages in the development of *E. histolytica* are still in doubt. Beyond a definite indication that the cysts hatch in the small intestine, and some inconclusive evidence that each liberates one 4-nucleate amoeba rather than four small uninucleate organisms, there is little to go upon. It is probable that the 4-nucleate amoebae divide into uninucleate individuals—if they really emerge in this condition: but whether the new individuals, however formed, are gametes which conjugate in pairs—as I suggested in *E. ranarum* (Dobell, 1909), and as Mercier (1910) claims to have shown in *E. blattae*—is still a matter for speculation.

Are there Races of E. histolytica *differing in Pathogenicity?*—It has frequently been suggested that there are races or strains of *E. histolytica* which differ in virulence or pathogenicity. This view is generally put forward owing to a misconception of the carrier condition, or through false analogies drawn between *E. histolytica* and the Bacteria. It merits some consideration here, however, as it involves several important points in the life-history of the parasite.

In the first place, it may be pointed out that the existence of pathogenic strains—causing amoebic dysentery, liver abscess, etc.—and non-

pathogenic strains—such as are seen in contact carriers—is, on *a priori* grounds, almost inconceivable. A "highly virulent" strain of the parasite, which always causes acute amoebic dysentery to its host, could not come into existence unless it simultaneously underwent a radical change in its life-cycle. Amoebic dysentery, as already noted, is disadvantageous to the parasite as much as to its host; for during a dysenteric attack the amoebae are cast out of the body and are unable to encyst. The "virulent strain," if it ever arose, would therefore be unable, in nature, to transmit itself beyond its first host. As I have elsewhere pointed out: "If the dysentery amoeba were always to cause acute dysentery in every human being it infected, it would become extinct within a period of time immeasurably less than that necessary for its extermination by any conceivable human agency" (Dobell, 1918*a*). It thus seems clear that "virulent" and "non-virulent" races of the parasite do not occur in nature.

The clinical differences between a healthy contact carrier of *E. histolytica* and a person suffering from acute amoebic dysentery are easily and simply explained if they are referred to differences in the susceptibility of the hosts. Some individuals are unable to tolerate the parasites, and react to their presence by developing acute dysentery; but such individuals are the rare exceptions, and the disharmony which results when they accidentally become infected is as disadvantageous for their parasites as it is for themselves. The ordinary individual, when he acquires an infection, becomes a carrier; and an equilibrium is at once established between his parasites and himself—a balance is struck between the regenerative powers of the host, and the destructive powers of the parasite, resulting in a condition which is not distinctly harmful to either.

That this explanation of the apparent differences in pathogenicity of *E. histolytica* in different hosts is the correct one, there is abundant evidence to show. We constantly see amoebic dysentery patients who make a complete clinical recovery without losing their infections. They become convalescent carriers indistinguishable clinically from contact carriers who have never had dysentery, though remaining still infected with the same strain of amoebae. But the cysts from the stools of such convalescent carriers, who are not suffering from dysentery, will, if swallowed by a susceptible host, again produce amoebae which cause dysentery. This experiment has been made on man by Walker, and has been frequently made with cats, which are so susceptible to the action of the parasite that they invariably acquire dysentery if they become infected. The cat never becomes a carrier, no matter what strain of amoebae is used to infect it; and consequently the infection in these animals can only be maintained by artificial means. Such experiments have been performed with the cat by many different workers, and there can be no doubt as to the facts. I may cite as a particularly instructive instance, however, an experiment made by Wenyon and O'Connor (1917), who gave a kitten a most acute and fatal dysentery by feeding it with cysts from the stools of a perfectly healthy man who had never himself had dysentery. Experiments such as this show clearly that it is the susceptibility or resistance of the host, and not a difference in the virulence of the parasite, which determines whether any given infected individual does or does not suffer from amoebic dysentery.

Equally conclusive experiments have been made upon man himself.

Walker (*vide* Walker and Sellards, 1913) infected a man by feeding him with cysts from the stools of another man—a convalescent carrier, who had previously suffered from amoebic dysentery. The second man, however, became a contact carrier. He acquired an infection with the same strain of amoebae, but did not develop dysentery. From the cysts in his stools a third man was then similarly infected. He also became a contact carrier. But from the cysts in his faeces a fourth man was infected, and he developed a typical attack of acute amoebic dysentery twenty days after ingesting the cysts. It is difficult to conceive of any more conclusive proof that it is the host, and not the parasite, which determines whether an infection is "pathogenic" or "non-pathogenic."

Baetjer and Sellards have recently attempted to show that there are strains of *E. histolytica* with different degrees of virulence.* Although this appears highly improbable, their experiments may be briefly noted here, as their conclusions have been accepted by some workers. The fullest account is given in the paper by Sellards and Baetjer (1915), in which three different "strains of *E. histolytica*" are described. The first was obtained from a patient (Case A) with intestinal symptoms but no dysentery. The amoebae in the stools were scanty, sluggish, and contained no red blood corpuscles. Their nuclear characters were not those typical of any of the intestinal amoebae of man.† "Cyst-like bodies" with 1, 2, or 3 nuclei were also found in the stools. A specimen containing chiefly the "cyst-like bodies" was injected into the caecum, ileum, and stomach of a kitten. Some weeks later the animal passed a few "amoebae" and "cysts" containing 4, 5, or 6 nuclei. These are all figured, and are strikingly like cells from the kitten's intestine, though unlike any developmental stages of *E. histolytica*. The kitten was killed and its "amoebae" inoculated into two others. One of these was also killed later, and "amoebae" were found in its large intestine *post mortem*. They were "not very well preserved, but apparently approached the histolytica type."

In the second case (Case B) amoebae "somewhat intermediate between the coli and the histolytica types" were found. No figures are given. A kitten inoculated with these "amoebae" developed dysentery, but recovered "before any entirely satisfactory specimens were obtained for morphological study." In the third case (Case C), no amoebae were ever found in the patient—only "cyst-like bodies containing from one to three nuclei." These were inoculated into the caecum and stomach of a kitten, which developed a watery diarrhoea a month later, with "amoebae" like those of Case A in its stools. Its symptoms abated, and it was finally killed. *Post mortem* neither amoebae nor evidences of amoebic infection were discoverable.

It will be obvious from this brief summary that there is not much evidence that Cases A, B, or C, or any of the experimentally "infected" kittens, ever harboured amoebae at all. Nothing greatly resembling *Entamoeba histolytica* is described or figured from any human case or kitten.

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* The authors consider that they were dealing with "atypical strains" of *E. histolytica* modified by environment.

† The authors only refer to *E. coli* and *E. histolytica*, and are apparently unaware that any other species occur in man.

Sellards and Baetjer believed that the atypical strains of amoebae which they studied produced atypical symptoms in their patients ; and that similar atypical infections with corresponding symptoms were produced in the experimentally infected kittens. Careful consideration of the recorded details of these experiments and the figures of their findings leaves me, however, in no doubt that they mistook cells of various sorts for amoebae and cysts. I can find no good evidence that amoebae of any sort were present in their patients or transmitted to their kittens, and it therefore seems superfluous to discuss whether their "atypical strains" were varieties of any particular species of *Entamoeba*. As they stand, their recorded observations supply no evidence whatever in support of the hypothesis that there are strains of *E. histolytica* which differ in virulence : and their hypothesis that strains producing atypical symptoms in man produce similar symptoms in experimentally inoculated kittens, is flatly contradicted by the well established fact that strains producing no symptoms in man produce the most acute dysentery in kittens. Indeed, every kitten which has ever been infected *per os* by means of the cysts of *E. histolytica* furnishes evidence against their view.

Can E. histolytica *live as a Commensal?*—There are still a few workers who find it difficult to believe that *E. histolytica* is a tissue-parasite always. They consider—if I understand them aright—that the ordinary healthy carrier of the parasite cannot have an ulcerated gut, because he manifests no clinical symptoms of disease : and they believe, apparently, that in healthy persons infected with *E. histolytica* the parasites must be living as harmless commensals—like *E. coli*. This view appears to me to be inconsistent with nearly every fact that is definitely known about *E. histolytica*. It is, in my opinion, unsupported by any concrete evidence, and seems to rest largely upon a misunderstanding of the carrier condition.

On purely *a priori* grounds the hypothesis is highly improbable. It is unlikely that an amoeba which is generally dependent upon living tissues for its nourishment should at times completely change its habits and become a feeder on bacteria. I know of no analogous instance in favour of such a supposition. Moreover, if it were proved that *E. histolytica* undergoes such radical changes in its habits from time to time, then the whole question of the pathogenicity of intestinal protozoa would require reconsideration. For example, there is no reason why *E. coli* should not also be able to undergo similar drastic changes in habit, and thus acquire pathogenic powers. But at present there is absolutely no evidence that either species is able to perform the remarkable transformations which the hypothesis demands of them.*

The hypothesis has been advanced especially by Kuenen and Swellengrebel (1913, 1914), and adopted by Woodcock (1917), Brug (1917 *b*), and a few other workers. Kuenen and Swellengrebel (1913) state that *E. histolytica* lives, during what they call its "*minuta* phase," as a saprozoic or commensal organism. It feeds on the gut contents, and not upon the tissues. Brug (1917 *b*) even asserts that the "*minuta*" forms are as "omnivorous" as *E. coli*. The only concrete evidence

* The hypothesis appears to me to rest partly on a false analogy with bacteria, as is shown by the fact that some writers speak of *E. histolytica* living "saprophytically"—a term improperly applied to anything which is not a plant.

which I can find for these and similar statements is, however, that given by Kuenen and Swellengrebel : and it appears to be contained in their statement that they have seen amoeboid forms of *E. histolytica* containing bacteria and other foreign bodies.

The explanation of this is very simple. In my experience *E. histolytica*, in a freshly passed stool, does not as a rule contain bacteria in its cytoplasm. This is true of all amoebae, whether large tissue-forms containing red corpuscles, or precystic individuals. The latter are, indeed, especially characterized by the absence of all ingested bodies from their cytoplasm. They are the forms which, far from ingesting solid bodies of any sort, have got rid of any inclusions which they may previously have contained (red corpuscles, fragments of tissue cells, etc.) in preparation for their encystation. They digest or egest food particles, and never ingest them. In this respect they resemble the encysting forms of *E. ranarum* and every other amoeba—both parasitic and free-living—with which I am acquainted. That bacteria are generally absent from these amoebae in a freshly passed stool is, I think, an indisputable fact. It is, however, equally true that bacteria can usually be found in *E. histolytica* amoebae, of every sort, in stools which are not fresh. In stale stools, or liver abscess pus, the majority of the amoebae often contain bacteria ; and as a rule the staler the material, and the more degenerate the amoebae in it, the more plentiful are the bacteria contained in them. A similar observation can be made on the amoebae in the tissues. In sections of ulcerated human intestines, which are very rarely obtainable immediately after death, most or all of the amoebae are usually degenerate : and many of them, as a rule, contain bacteria in greater or less numbers. If a cat with acute amoebic dysentery is killed, and its intestine fixed immediately by a good cytological method, and then sectioned, as a rule not a single amoeba will be found to contain bacteria. Although these are abundant in the gut contents and the older necrotic tissues, the areas occupied by the amoebae, no less than the amoebae themselves, are remarkably free from bacteria of every sort. If, however, the infected cat is allowed to lie dead for some time before its tissues are fixed, then the amoebae in the ulcers will often be found subsequently to contain bacteria. In such circumstances the amoebae are always more or less degenerate, and all more or less full of bacteria—exactly as they are in human tissues obtained *post mortem*.

Examination of really fresh material will convince anybody, I think, that *E. histolytica*, when in the amoeboid state, normally never contains bacteria. Since these only appear in the amoebae when they are degenerating or dead, the most reasonable way to account for this is, obviously, to infer that dead and dying amoebae are subject to bacterial invasion. Cells and dead protoplasm of all sorts, when present in the gut contents, are readily invaded by bacteria ; and there is no obvious reason why amoebae should not share the same fate. I have not the slightest doubt that this is usually the correct explanation of the fact that *E. histolytica* amoebae sometimes, and in certain circumstances, contain bacteria.

When individuals of *E. histolytica* are found to contain bacteria, then careful examination always shows that the amoebae are degenerate. Precystic amoebae, for example, containing numerous bacteria, are very common in stools which are not perfectly fresh ; but as a rule every such

individual will be found to have an abnormal nucleus, and to show other typical signs of degeneration. Fig. 81 (Pl. V) depicts a common type of precystic amoeba from a stale stool. The structure of its nucleus at once stamps it as a dying or dead organism.

E. histolytica may also—though this is very rare—be parasitized by bacteria. I have made a careful study of three such infections, and have satisfied myself of the correctness of the observations. I do not know whether the parasitic bacteria occur in the tissue-invading forms within the intestinal ulcers: but they are certainly present in the precystic amoebae. Fig. 83 (Pl. V) shows one of these from the case which I studied in the greatest detail. The patient in whom this infection occurred was a convalescent carrier of *E. histolytica*, and the amoebae were obtained by the administration of a saline purgative. The preparations were fixed almost immediately after the amoebae left their host, so that a *post mortem* invasion of their protoplasm by bacteria can, I think, be excluded. A large percentage of the amoebae, in this instance, contained the micro-organisms shown. They were also present in the cysts (fig. 84, Pl. V); but almost all such infected cysts were uninucleate. There were uninfected amoebae also, and normal uninfected cysts containing 1, 2, or 4 nuclei. It thus seems highly probable that the parasitic bacteria in the precystic amoebae did not prevent encystation, but arrested the development of the cysts at the uninucleate stage. The bacteria were easily visible inside the living uninucleate cysts; and when these were kept they rapidly degenerated—the bacteria apparently increasing in numbers within the cysts, which finally contained merely disintegrated protoplasm and many bacteria in active Brownian movement. All the parasitized amoebae and cysts showed more or less degenerate nuclei (cf. figs. 83 and 84).

Another case of the same sort I studied with Dr. A. C. Stevenson, who kindly permits me to record our observations. This patient also was a convalescent carrier, and was under observation for about six months, during which time his stools were examined repeatedly. They almost always contained numerous cysts of *E. histolytica;* and these were almost invariably uninucleate, and parasitized by bacteria (fig. 85, Pl. V). They frequently contained chromatoid bodies also, but these were generally large and easily distinguishable from the bacterial inclusions. Binucleate cysts were very rare, and 4-nucleate cysts were never found. As in the preceding case, the cysts died very rapidly outside the body on every occasion when they were kept under observation. We came to the conclusion that the cysts were infected with a parasitic micro-organism which inhibited their development, but we did not obtain amoebae from this case. Infected cysts were passed by this patient for about five months, during which time he was unsuccessfully treated several times. At each relapse after treatment he passed infected uninucleate cysts once more. As only degenerate uninucleate cysts occurred, we were often in doubt as to whether many of them were really cysts of *E. coli.* After a final thorough course of treatment, however, with emetine bismuth iodide, all the cysts vanished from the stools and never reappeared subsequently, although the examinations were continued over a considerable period.

The first patient was also treated with emetine bismuth iodide, and likewise cured of his infection. Both cases were, I think, undoubtedly infected with a strain of *E. histolytica* which was itself infected with a

bacterial parasite. In both these cases, and at least one other which I have seen, the parasitic bacteria appeared to be identical. They were cocco-bacilli of the form shown in figs. 83-85, Pl. V.

I would also note here that the chromatoid bodies of *E. histolytica* may be mistaken for bacteria when, as sometimes happens, they are in the form of slender rods, filaments, or small granules. As noted elsewhere, these bodies may occur not only in the cysts but also in the precystic amoebae (fig. 82, Pl. V) ; and such amoebae may easily be misinterpreted as precystic individuals containing ingested bacteria.

When precystic or other amoeboid forms of *E. histolytica* contain "ingested bacteria" it is therefore necessary to prove (1) that these are not really chromatoid bodies, or, if really bacteria, (2) that they are not parasites or (3) organisms which have invaded the dead or dying amoebae. I cannot find, however, that Kuenen and Swellengrebel, or any other workers who share their view, have attempted to exclude any of these sources of fallacy. Until they do, their statement that *E. histolytica* can feed upon bacteria is not supported by any evidence. The mere finding of "bacteria" in amoebae by no means proves that *E. histolytica* is capable of living as a commensal.

Kuenen and Swellengrebel (1913) also describe a case in which they found "*minuta*" forms of *E. histolytica* containing starch grains, in addition to bacteria. At first sight this appears puzzling. But the explanation is at once found when, a little later, we read that this case also passed 8-nucleate cysts of *E. histolytica*. I have not the slightest doubt that this case was really infected with *E. coli*, and that the small amoebae containing starch grains and bacteria, and the 8-nucleate cysts, all really belonged to this species. To the three postulates made in the previous paragraph it thus seems necessary to add a fourth—that when, in a stool, "amoebae" are found containing bacteria, some proof that they belong to *E. histolytica* shall be required.

From the foregoing considerations it therefore appears to me highly improbable that *E. histolytica* can alter its mode of life so fundamentally as to become a mere commensal like *E. coli :* and in support of such a view—which is entirely opposed to my own experience, and to me almost inconceivable—I can find no evidence whatsoever.

Sexual Phenomena. Conjugation.—No conjugation of any sort has been shown to occur at any stage in the life-history of *E. histolytica*. It is sometimes stated that Schaudinn (1903) described an autogamy in the cysts of this species : and although this is incorrect, such a process was actually described by Hartmann (1908) in "*E. tetragena*"—really the same species. As I pointed out at the time (Dobell, 1909), his interpretation was not justified, and the alleged "autogamy" was disproved later by Walker* (1911).

Sexual stages in the life-history have been suggested by several workers, but are still unsupported by evidence. Job and Hirtzmann

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* Walker gives me the credit of having pointed out that the development in the cysts is by "straightforward nuclear division" : but he is not quite correct in stating that I came to this conclusion in the case of *E. coli*. I proved it in *E. ranarum*, and pointed out that the development of *E. histolytica* (then called "*E. tetragena*") was probably "almost identical." At that time I was still in doubt about the development of *E. coli*, owing to the very definite statements of Schaudinn (1903), and Wenyon's (1907) "confirmation" in *E. muris*.

(1916) describe a stage which they believe to be "a female gamete"; but they observed no conjugation or other sexual process, and no reasons are given for their peculiar interpretation. Mathis and Mercier (1916 *a*, 1917 *b*) have asserted that there are two different kinds of cysts in this species—"microcysts," measuring 12·5 μ in diameter, and "macrocysts" of 14 μ: and they suppose that these, when ingested, liberate microgametes and macrogametes respectively, which conjugate with one another. It has been shown (Dobell and Jepps (1918); Malins Smith (1918)) that this dimorphism of the cysts is not really observable; and consequently the rest of their hypothesis is deprived of its chief evidence.

It is not impossible, however, that conjugation does occur at the stage in the life-cycle suggested by Mathis and Mercier — namely between the amoebae liberated from the cysts in the small intestine at the beginning of their life-history. Nevertheless, this has yet to be observed. I originally suggested (1909) that such a development might occur in the very closely related species *E. ranarum*, and Mercier (1910) has since described it in *E. blattae*. But his observations have not yet been confirmed, and at present there is no proof that conjugation occurs in any other parasitic amoeba.

Other Interpretations of the Life-history.—It is now generally recognized that the life-history of *E. histolytica* follows the course here described. Since the appearance of the works of Walker (1911, 1913), any other interpretation of the development of this parasite has, I think, become untenable. My own experience has, at all events, convinced me over and over again that his conception of the life-cycle as a whole is the only correct one. It agrees entirely, moreover, with that of *E. ranarum*, which I had worked out previously. In both species there is a large amoeboid form which represents the ordinary vegetative and reproductive stage. In certain circumstances this form produces—probably by simple division—a smaller precystic form which does not feed in the usual way, but gets rid of any food it may happen to contain, and then encysts. Within the cyst a nuclear multiplication occurs, by successive divisions, until four nuclei are formed. The cyst is then mature, and ready to infect a fresh host. The whole life-history is extremely simple, and is only complicated, in *E. histolytica*, by the occasional wandering of the parasite from its normal habitat in the gut wall into other tissues. The chief difference between *E. ranarum* and *E. histolytica* is in habit—the one being a simple commensal, the other a true parasite, incapable of nourishing itself upon anything but living tissue.

Several other views have been advanced concerning the life-history of *E. histolytica*. Schaudinn's (1903) description is now universally admitted to have been incorrect, and merits no further discussion. Kuenen and Swellengrebel (1913, 1914), among more recent workers, hold peculiar views concerning the life-history of *E. histolytica*. They believe (*vide* Kuenen and Swellengrebel, 1913) that the parasite, which they call—without any justification—*E. tetragena*, occurs in three "phases": a "*histolytica* phase," in the tissues in dysentery, liver abscess, etc.; a "*minuta* phase," living as a harmless commensal like *E. coli* in the lumen of the gut; and a "*tetragena* phase" in which the parasite appears in the encysted form. They consider that the "*minuta* phase," which is saprozoic, can "reproduce" the "*histolytica* phase," which is parasitic in the tissues, and *vice versa*. They do not regard the "*minuta*" forms as precystic amoebae; though—if I understand them correctly—they believe

that the "*minuta*" amoebae give rise, in some way, to the cystic "*tetragena* phase." Swellengrebel and Schiess (1917) even say that this peculiar development has been "demonstrated"; but it appears to me to rest upon a misconception of the part played by the precystic amoebae in the life-cycle of the parasite.

As we have seen,* Kuenen and Swellengrebel (1913) have never proved that the "*minuta*" forms are capable of living saprozoically, and it is more than probable that they are not. So far as I understand their views, they rest upon a misinterpretation of the following facts, which are well known to everybody familiar with *E. histolytica*. (1) A patient suffering from acute amoebic dysentery passes the large tissue-invading forms of the parasite only (their "*histolytica* phase"), and not precystic amoebae or cysts. (2) When the symptoms abate, the patient suffers from diarrhoea, and passes chiefly precystic amoebae (their "*minuta* phase"). (3) When the symptoms disappear, and the patient's stools become solid, he passes cysts only (their "*tetragena* phase"). All these events are very simply explained—as I have already shown—without having recourse to their hypothesis. In the ordinary carrier of *E. histolytica* all the "phases" actually exist simultaneously. The cysts are in his stools; the precystic amoebae are in the lumen of his gut—from which they can easily be obtained at any time by the administration of a purgative; and the tissue-invading forms are in the ulcers in his gut wall—from which they could be obtained by scraping the ulcers, though not, as a rule, by the administration of purgatives. I do not know how Kuenen and Swellengrebel account for the appearance of large numbers of cysts in the faeces of a carrier every day for long periods, unless they suppose that the cysts multiply in the large intestine: nor how they would account for the fact that the carrier has an ulcerated intestine containing parasites of "*histolytica*" form; nor yet how they would explain the case of a carrier with "*tetragena* phase" cysts in his faeces, an abscess full of "*histolytica* phase" amoebae in his liver, and "*minuta* phase" parasites discoverable in his stools as often as a purgative is administered to him. But their hypothesis will require much further elaboration and far more evidence than they have hitherto adduced if it is to explain the facts and gain any adherents. At present I cannot find any evidence in favour of it in the publications of Kuenen and Swellengrebel (1913, 1914), Swellengrebel and Schiess (1917), or any other workers.

Another peculiar interpretation of the life-cycle of *E. histolytica* is that put forward by Mathis and Mercier (1916). These authors, who call the parasite *E. dysenteriae*, believe that it occurs in three chief "forms" or "types,"—a "*tetragena* form," free in the gut; an encysted form, in normal stools; and a "*histolytica* form" in dysenteric stools. Of the last they say: "The fact that the *histolytica* type is seen exclusively in the bloody mucous stools of acute attacks of amoebiasis, allows us to admit that this type does not belong to the developmental cycle of the parasite." And they conclude—if I understand them correctly—that the ordinary forms of *E. histolytica*, which occur in all the tissues capable of invasion, and which constitute the major part of the species, are a kind of developmental abnormality. Their reasons for taking this curious view are not clear to me: and consequently, beyond noting that their use of the terms "*histolytica*" and "*tetragena*" seem to have but little historic

* See p. 61 *et seq.*

justification, and are therefore confusing, I am unable to criticize their conceptions. I find nothing to support their view, and do not understand why they do not adopt the simple, obvious, straightforward, and consistent interpretation of Walker and most other workers.

Animal Infections.—Entamoeba histolytica is the only amoeba which has been proved to be able to live in more than one host. Man is its normal host, in all probability, but other animals can be experimentally infected. Lösch (1875), as already noted, infected a dog; and Hlava (1887), Kruse and Pasquale (1894), Harris (1901), and Dale and Dobell (1917) also succeeded in infecting this animal. The animal most readily infected is, however, the cat, to which the amoeba has been successfully transmitted by a large number of workers.* The earlier workers infected cats by injecting amoebae from human dysenteric stools into the large intestine *per anum*, and this method is frequently successful. They found, as was to be expected, that no infection took place if the amoebae were administered *per os*—a fact often since confirmed. Quincke and Roos (1893) found, however, that cats can be infected by causing them to swallow the cysts of *E. histolytica*, and this too has been frequently confirmed by later workers.† There is no good evidence that the cat can be infected in other ways.‡

The cat, when infected, typically aquires acute and fatal amoebic dysentery, closely resembling the comparable condition seen in man. Spontaneous recovery takes place very exceptionally, and the cat, in all probability, never becomes a carrier. The parasite appears to be incapable of forming cysts in the cat's intestine (cf. Dale and Dobell, 1917). The "cysts" described in cats by several authors were almost certainly not cysts of *E. histolytica*, for no competent workers who have studied the infections in cats have ever seen cyst-formation in this animal. There is already a large amount of evidence on this head. For example, Dale and I (1917) studied about 150 kittens infected with *E. histolytica*, but in spite of the most careful search never discovered any cysts in them. This is in complete agreement with the observations of Wenyon (1912) and many other careful workers.

Darling (1913 *c*) found "uninucleate cysts" in an infected kitten, but it is highly probable that they were merely rounded amoebae. The "cysts" of *E. histolytica* observed in cats by Sellards and Baetjer (1915) were obviously cells. Their description and figures make this clear—their "cysts" being quite unlike those of *E. histolytica*, and containing sometimes 5 or 6 nuclei. In an earlier paper (Baetjer and Sellards, 1914) they stated that "encystment frequently occurred" in their infected cats, but advanced no evidence. They have also stated (Baetjer and Sellards, 1914*a*) that they produced "a carrier state" in cats; but they

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* See, for example, Hlava (1887), Kartulis (1891), Kovács (1892), Quincke and Roos (1893), Kruse and Pasquale (1894), Jürgens (1902), Wenyon (1912), Darling (1913*a*), Baetjer and Sellards (1914), Dale and Dobell (1917), etc.

† Cf. Huber (1903, 1909), Kuenen and Swellengrebel (1913), Wenyon and O'Connor (1917), Dale and Dobell (1917), etc.

‡ The fantastic experiments of Lesage (1907 *a*) are hardly worthy of mention. He claimed to have infected cats by placing stale dysenteric stools or liver abscess pus up their noses, by injecting similar material hypodermically, by putting the dried blood of an infected cat into the nose of another, and by other extravagantly impossible methods.

appear to mean by this that they simply observed "periods of apparent health alternating with acute relapses"—a very different thing from the true carrier condition seen in man.

Cutler (1918) somewhat casually mentions that he found "a few cysts" in two experimental cats (Nos. 1 and 2), although his account of the second animal makes it probable that it was never really infected with *E. histolytica*. In a more recent paper (Cutler, 1919) he says: "in the cat's gut cyst formation probably never occurs" (p. 131); again, "in cat infections, . . . cyst formation . . . is rare" (p. 140); and again, "If Dale and Dobell mean . . . that they found no evidence of cysts (*sic*) formation in cats, I am in entire agreement." Cutler has thus twice asserted and twice denied that cysts are formed in the cat. This seems to me sufficiently contradictory to render further criticism superfluous.

Amoebic abscess of the liver has been produced both in dogs and in cats, as a sequel to intestinal infection. It was first produced experimentally in the dog by Harris (1901), and Kartulis (1913) has recorded a spontaneous case. Craig (1905), Werner (1908), Huber (1909), Wenyon (1912), Dale and Dobell (1917), and others, have observed amoebic liver abscesses in cats. The first case was probably Huber's, though not the first to be described. Kartulis (1913) states that he has seen a case of spontaneous amoebic dysentery and liver abscess in a badger, and this may have been due to accidental infection with *E. histolytica*. In the cat, the amoebic liver abscesses closely resemble the early abscesses seen in man.

Although carnivores seem most easily infected with *E. histolytica*, there is now evidence that rodents can also harbour the parasites. The most interesting case is that of the guinea-pig. Kartulis (1886), Hlava (1887), Kruse and Pasquale (1894), and Werner (1908), were unable to infect this animal; but Baetjer and Sellards (1914*a*) and Chatton (1917*a*, 1918, 1918*b*), have since succeeded. From the observations of these workers it appears that *E. histolytica* infection in the guinea-pig is not accompanied by dysentery. It is sharply localized in the caecum, where it gives rise to remarkable lesions resembling neoplasms, and described by Chatton as a "lympho-sarcomatoid hyperplasia." The infection can be brought about by cysts *per os* or by amoebae *per anum* (Chatton), as in the cat, and it appears to be usually fatal to the guinea-pig. A fuller account will be found in the papers cited. It only remains to add that "amoebiasis" in the guinea-pig appears to be a disease of a type different from the dysenteric infections of man and carnivores. Whether it is ever paralleled in human beings remains to be seen. Possibly some cases of "latent amoebiasis" in man are of a similar character. No cysts of *E. histolytica* have yet been described, however, from the faeces of infected guinea-pigs. And it is worthy of note that this animal has an amoeba * of its own, resembling *E. coli* and *E. muris*. Leger (1918) has recently given an account of an epizootic, believed to be due to intestinal amoebae, among guinea-pigs in Cayenne (Guiana). The reasons for connecting the amoebae with the disease are, however, far from obvious, and certainly inconclusive.

Kartulis (1886), Hlava (1887), Dale and Dobell (1917), and others

* Cf. Chatton (1917*a*), etc. This organism was probably first noted by Walker (1908). Its correct name appears to be *Entamoeba cobayae* Walker emend. Chatton (syn. *E. caviae* Chatton, 1918 *b*)—if really a distinct species.

have unsuccessfully attempted to infect the rabbit. But Huber (1909) claims to have succeeded, and his observations, though little noticed, are of considerable interest. He says he succeeded in infecting 4 out of 8 rabbits fed on human stools containing cysts of *E. histolytica*. They did not acquire dysentery or diarrhoea, nor did they pass amoebae with their faeces. Three died after 3—5 weeks, and the fourth was killed. *Post mortem* examination showed that the amoebae were localized in ulcers in the caecum. The ulceration was characterized by great inflammatory thickening of the submucosa, and was different from that usually seen in man and the cat. It would be interesting to know whether the rabbit can become a carrier of *E. histolytica*—like a human being; and Huber's results seem to indicate the importance of fuller inquiry.

Unsuccessful attempts have been recorded to infect rats (Werner (1908), Dale and Dobell (1917), Chatton (1918*b*), etc.); mice (Chatton, 1917*b*, 1918*b*); and a gerbil (Kruse and Pasquale, 1894). Lynch (1915*b*), however, claims to have succeeded in the case of the rat, and also to have observed spontaneous amoebic dysentery in this animal. Although he gives a circumstantial account of the lesions, and appears to be in no doubt about the identity of his amoebae with *E. histolytica*, his description of the parasites is very unconvincing. He seems, moreover, to be unaware that rats are sometimes infected with another species of amoeba (*E. muris* ?), which may possibly account for his findings—as Chatton (1917*a*) has pointed out. Lynch's conclusions can hardly be accepted without further evidence. Kartulis (1891), it may be added, had previously stated that rats may suffer from spontaneous amoebic dysentery.

Experiments on monkeys, and the amoebae naturally occurring in these animals, will be considered in another place. (*Vide* p. 131 *infra*.)

Dissemination.—It is clear that *E. histolytica* infection is normally conveyed from man to man by the contamination of food or drink with faecal matter containing the cysts of the parasite. The spread of infection must always depend, therefore, upon defective sanitary conditions—as is verified by the evidence which has accumulated showing that infections with this organism are commonest in the tropics and other places where hygienic conditions are worst. Infection is probably acquired in many cases by drinking water polluted with faeces containing cysts of *E. histolytica*. It has also been shown by Wenyon and O'Connor (1916, 1917), that flies may act as spreaders of infection: for flies will feed readily upon human faeces containing cysts, and can pass these intact through their bodies and out in their own faeces. It thus seems probable that the fly is often an important agent in spreading *E. histolytica* in nature. It should be noted, however, that some other workers have regarded the activities of the fly in this connexion from a different standpoint. Roubaud (1918) suggests, indeed, that the fly is beneficial rather than harmful: for its faeces rapidly undergo desiccation when deposited; and any cysts which it may pass are thus, in ordinary circumstances, rapidly destroyed.

Cultivation.—Many workers have, in the past, attempted to cultivate *E. histolytica*. The earlier of them often claimed to have succeeded, but it is now generally recognized that they merely cultivated free-living amoebae from the stools. The original claim of Kartulis (1891) was disposed of by Celli and Fiocca (1894, 1894*a*, 1895) and Casagrandi and Barbagallo (1895*a*, 1897, 1897*a*), who arrived at the true explanation of

his observations; but since then history has repeated itself many times.* The conclusive experiments of Walker and Sellards (1913) finally placed the matter on a thoroughly scientific basis, and during the last decade claims to have cultivated any parasitic amoeba have become fewer and fewer. There are probably very few workers now who believe that *E. histolytica* is cultivable by any known method.

Recently Cutler (1918) has published a description of experiments which lead him to believe that he has discovered methods for the cultivation of this parasite. His work therefore requires notice here. Although his account appears at first sight to prove that his claims are justified, they have not yet been confirmed by other workers. In conjunction with Capt. S. R. Douglas, I.M.S. (ret.), I attempted to cultivate *E. histolytica* by his method, but all our attempts were complete failures. This work, in conjunction with further information concerning his methods kindly given to me subsequently by Mr. Cutler himself, has shown me that his observations may be capable of a different interpretation from that which he has put upon them. His experiments were not quite so simple as they seem from his brief account; and consequently I cannot yet regard his claim as fully justified until further evidence and explanations, with independent confirmation by others, are forthcoming.

Treatment.—It is not my intention to discuss the treatment of *E. histolytica* infections here: but one aspect of this subject is so remarkable that it is impossible to omit all reference to it even in a purely zoological memoir. I refer, of course, to the specific action of emetine on human infections with *E. histolytica*. Ipecacuanha has been used for centuries as a cure for dysentery, but it is only comparatively recently that its alkaloids—especially emetine—have been proved to be of the highest therapeutic value in the treatment of infections with *E. histolytica*. Upon human infections with this amoeba emetine has a remarkably specific action. It was originally thought, as a result of Vedder's (1912) observations, that emetine is a peculiarly "amoebicidal" substance, and that it acts by killing the parasite directly when administered to an infected human being. It is almost certain now, however, that the alkaloid is not particularly poisonous to amoebae, and that its action is primarily upon the host and not upon the parasite (Dale and Dobell, 1917). Whatever the mechanism of this action may be, there can be no doubt that emetine has a truly remarkable therapeutic efficacy when administered in a suitable manner;† and this specific reaction of the parasite to the drug has done much to clear up the problem of the species of amoebae inhabiting the human bowel.

Geographical Distribution.—It is now certain that the present geographical distribution of *E. histolytica* is world-wide. It is not restricted to the tropics—as is frequently assumed—but occurs in every country in which it has been sought by competent observers. Indigenous infections are now known to occur, for example, in Russia (Lösch, 1875; Yakimoff, 1917); in France (Gailliard and Brumpt (1912), Paviot and Garin (1913), Landouzy and Debré (1914), Ravaut and Krolunitzki (1916), etc.); in Germany (Jürgens, 1906); in the Northern United

* Cf. Musgrave and Clegg (1904, 1906), Lesage (1905, 1907), Walker (1908), Noc (1909), Gauducheau (1912), etc.

† For further information on this subject see especially Wenyon and O'Connor (1917), Dobell (1916, 1917), Dobell, Gettings, Jepps, and Stephens (1918).

States of America (Giffin, 1913); and in the British Isles (Yorke, Carter, Mackinnon, Matthews, and Smith (1917), Laidlaw (1918), Matthews and Smith (1919), Baylis (1919), etc.)—in addition to all the tropical and subtropical countries where its occurrence, as known from the diseases which it produces, has long been famous. It is hardly possible to doubt that *E. histolytica* occurs wherever man occurs—though the frequency of infection is probably not equal in all places and among different populations.*

(2) *ENTAMOEBA COLI* (GRASSI, 1879) CASAGRANDI & BARBAGALLO, 1895 (*NEC* LÖSCH, 1875).

"Amoebae" Lewis, 1870.
"Amoebae" Cunningham, 1871.
"Psorospermi" Grassi, 1879 (*pro parte*).
Amoeba coli Grassi, 1879 (*nec* Lösch, 1875).
Protomyxomyces coprinarius Cunningham, 1881 (*pro parte*).
? *Amoeba intestinalis* Blanchard, 1889.
Amoeba coli mitis
Amoeba intestini vulgaris } Quincke & Roos, 1893.
Entamoeba coli Casagrandi & Barbagallo, 1895.
Entamoeba hominis Casagrandi & Barbagallo, 1897.
Entamoeba coli Schaudinn, 1903 (*nec* Lösch, 1875).
"Entamoeba Loeschi" Lesage, 1908.
Amoeba coli Brumpt, 1910 (*nec* Lösch, 1875).
Entamoeba williamsi Prowazek, 1911.
Entamoeba hartmanni Prowazek, 1912 (*pro parte*).
Entamoeba brasiliensis Aragão, 1912 (*pro parte*).
Löschia coli Chatton & Lalung-Bonnaire, 1912.
Entamoeba coli communis Knowles & Cole, 1917 (*pro parte*).
Endameba intestinivulgaris Aragão, 1917.
Endamoeba intestino-vulgaris Aragão, 1917.
Endameba coli Craig, 1917.
Endameba hominis Pestana, 1917.

History and Nomenclature.

The species here named *Entamoeba coli* was probably discovered by Lewis (1870) in India. He saw "amoebae" in the stools of patients suffering from cholera, but from his account it is impossible to identify their species with certainty. At about the same time, however, his collaborator Cunningham (1871) made similar observations; and from the latter's publications it is possible to identify their findings with some certainty. Cunningham (1871) says that he found "amoebae" in 18 per cent. of the choleraic stools which he examined in Calcutta; and as he has carefully noted some of their characters, there can be no doubt that what he called "amoebae" really were these organisms—not cells or

* It seems equally certain that there is only one species of amoeba responsible for all the amoebic diseases—dysentery, liver abscess, etc.—all the world over. At all events, no amoeba other than *E. histolytica* has ever been proved to be pathogenic to man

other protozoa. Moreover, it is clear that the amoebae which he observed were intestinal amoebae, and not free-living species. These are probably the first recorded observations on the intestinal amoebae of man; and as Cunningham's early work has hitherto received but scant attention,* and is not easily accessible, I shall consider it in some detail.

Cunningham was fully alive to the possible errors involved in examining stools for amoebae. "There is," he writes, "a considerable amount of difficulty and numerous sources of fallacy to be encountered in proceeding to the consideration of *amoebae*" (1871, p. 44)—a remark which his successors might have taken to heart with much profit to science and themselves. He notes further that he distinguished his amoebae from cells in the stools by their "power of free progression," and he observes that they rapidly die outside the human body. He speaks of both free and "encysted" amoebae, but most of the latter—though probably not all—were merely rounded and motionless individuals. For the first time he observed and recorded that the intestinal amoebae differ from free-living species in possessing no "contractile vesicles." It is clear from his later work—though not from the earlier—that Cunningham (1881) saw both the free forms and the cysts of *Entamoeba coli*. "They occur in the excreta during health, as well as in cases of cholera and other morbid conditions affecting the intestinal canal" (Cunningham (1881), p. 248). His text-fig. 4 depicts the cysts, and text-figs. 5 and 6 show the free amoebae—the last being an unmistakable *E. coli* containing ingested *Blastocystis* ("sporoid bodies"). "In the encysted condition, when their form is more or less spherical or elliptical, they frequently attain a diameter of 25 μ or even more, and they may range downward from this until their diameter only amounts to 8 μ" (p. 249). From the last remark it seems probable that he observed the cysts of more than one species. He notes the variable size and shape of the amoebae, the "changeable vacuoles, often of considerable size," and again the absence of a contractile vacuole. Of the nucleus he says that it was not always visible, that it "may or may not include a visible nucleolus," that it may attain a diameter of 7-9 μ; and that "it is circular and apparently discoid, but in some cases may appear annular from the presence of a thickened margin" (p. 249). I believe nobody who reads Cunningham's account and studies his pictures can fail, if he knows this organism, to recognize in them the commonest of the intestinal amoebae of man—*Entamoeba coli*.

Unfortunately, in his later work Cunningham was led astray by the flagellates and other organisms which developed in his material: and he combined together all the organisms which he found in faeces—including the intestinal amoebae and flagellates, which he had accurately observed—to form the life-history of a single organism which he named "*Protomyxomyces coprinarius*," and regarded as a sort of Mycetozoon.

After Cunningham, Grassi (1879*a*) described amoebae from human faeces, and identified them with the organisms then recently discovered by Lösch (1875). Accordingly, he named them "*Amoeba coli* Lösch." Now there can be little doubt that Grassi was mistaken in this. The

* Craig (1908) has stated that Grassi (1888) "was probably the first investigator to demonstrate the occurrence of amoebae in the faeces of healthy individuals" (p. 332); and he adds that Cunningham "was probably observing developmental stages of flagellates." Few readers of Cunningham's works are likely to share these views.

organisms which he found were, for the most part, *Entamoeba coli*—the same as the amoebae observed by Cunningham : and they were not Lösch's "Amoeba coli," which was *Entamoeba histolytica*. This wrong identification has been responsible for much of the confusion which arose later. Grassi, moreover, not only saw *E. coli* in the amoeboid stage : he also saw its cysts. But he mistook these for coccidia,* and described them as such (Grassi, 1879). His figures (1879, figs. 10 and 11) are conclusive in this respect. Later, he himself confessed that his "coccidia" were "resting amoebae," or cysts (Grassi, 1882, 1883, 1888). Grassi's second account (1882, 1883) of his "*Amoeba coli*" is brief, and is accompanied by an unrecognizable figure of the organism. He says it occurs in healthy people, as well as in those suffering from diarrhoea or other intestinal ailments : but as it lives in the more fluid part of the contents of the colon, it is most abundant in the stools when these are soft or diarrhoeic. With Cunningham he regarded the organism as non-pathogenic. It was stated to measure 8-22 μ in diameter, its nucleus being 2.2—5.5 μ and round in shape, "with 2 (? always) nucleoli." The ectoplasm was said to be very thin, the endoplasm granular and containing numerous vacuoles filled with food of all sorts—starch grains, bacteria, etc.

In a later paper Grassi (1888) gives some further details concerning his "*Amoeba coli*." He says the organism encysts in the same way as *Endamoeba blattae*.† The cysts are smaller than the amoebae ; they contain "more or less numerous (3, 6, 9) nuclei," and are diagnostic of the species. He adds the important statement that he and Calandruccio —with whose collaboration the above observations were made—have shown "by repeated experiments" that human beings, when they swallow the cysts, acquire infection with the amoebae, which multiply by fission in their new host. Calandruccio (1890) has confirmed these statements. Grassi (1888*a*) reaffirms his belief that the amoebae are harmless to man—"they are simple commensals, altogether innocuous." He says he has found them present in enormous numbers in the stools not only of persons suffering from dysentery, but also of those with many other disorders—typhoid, cholera, pellagra, simple diarrhoea *ab ingestis*, etc.—and in the stools of perfectly healthy people, who often continue for months to pass the cysts in large numbers. The infection experiments noted above were not followed by dysentery or other intestinal derangement.

From the foregoing it is abundantly clear that Grassi studied chiefly the amoeba here described as *Entamoeba coli*, though he did not count the nuclei in the cysts correctly—the numbers he gives‡ being very unusual, and the typical number (8) in the mature cyst not being mentioned. It should be noted that Grassi mentions (1883, 1888*a*) that his "*Amoeba coli*" will ingest red blood corpuscles, when these are present in the intestinal contents. It thus seems probable that he also saw *E. histolytica*, but did not distinguish it from the commoner form.

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* For further details concerning Grassi's "coccidia" see my paper on the coccidia of man (Dobell, 1919).

† Grassi (1888) incorrectly called this organism "*Amoeba blattarum*."

‡ Calandruccio's (1890) statements concerning the number of nuclei in the cysts of *E. coli* are identical with Grassi's.

I therefore conclude that Grassi's "*Amoeba coli*" was, for the most part, *Entamoeba coli;* but that it included also, in all probability, some individuals at least of *E. histolytica.*

The next important work to be considered is that of Quincke and Roos (1893) and Roos (1894), whose admirable work on *E. histolytica* has already been noted. These observers also studied *Entamoeba coli*, of which they gave an easily recognizable description and good figures. They found the organism in the stools of a patient with colitis, and in 9 healthy human beings out of 24 whose stools they investigated. Unfortunately, the clinical condition of the first case led them to believe that his amoebae were a distinct species from the others, though they found no morphological differences in either the amoebae or their cysts to justify this conclusion. They named this "pathogenic" form "Amoeba coli mitis," and the form from healthy people "Amoeba intestini vulgaris." From the descriptions and figures these amoebae were identical, except as regards their provenance ; and both these names were therefore given to the same species—namely, *Entamoeba coli.* I would point out here again that the names which Quincke and Roos gave to their amoebae should not be considered in discussing priority in the nomenclature of these organisms. The names are trinominal descriptive terms, and not binominal names bestowed in accordance with the Rules of Nomenclature. They have no status and are not valid zoological names. It is therefore not justifiable to attempt to revive them, as Aragão has recently done. He proposes (1917) to replace the name *E. coli* by "*Endameba intestinivulgaris*" or (1917 *a*) "*Endamoeba intestino-vulgaris* Quincke and Roos, 1893"; though these are not their names at all, but Aragão's emendations which attempt to make one of their designations of this organism conform to the Linnaean system—a system which they did not follow. Aragão's names are thus synonyms without validity.

Quincke and Roos gave a good description of *E. coli*, and of the characters which distinguish this species from *E. histolytica.* They describe the amoeba as sluggish, with no sharp demarcation between ectoplasm and endoplasm, with food-vacuoles containing many ingested foreign bodies but never red corpuscles. The nucleus is also noted and recognizably figured. The cysts are distinguished from those of *E. histolytica* by their larger size (16—17 μ) and thicker walls, and are said to contain one or more nuclei. On this point their description is defective. They found further that this species is non-pathogenic to the cat, and they did not succeed in infecting this animal either with cysts *per os* or with amoebae *per anum :* but Roos (1894) points out that man probably acquires his infection by swallowing the cysts, in the same way that the cat may become infected with *E. histolytica*—as their experiments had proved. It is difficult to understand why these really excellent observations should have had so little influence on later workers. Their only real blemishes were errors in nomenclature and insufficient investigation of cytological details.

Soon after Quincke and Roos, Casagrandi and Barbagallo (1895, 1897) made important contributions to our knowledge of *Entamoeba coli.* Unfortunately they contradicted the conclusions of the former workers—without adequate evidence—and maintained that there is only one species of intestinal amoeba in man. They maintained—with Grassi—that this amoeba is harmless, and occurs in both dysenteric

and non-dysenteric persons. Further, they identified the organism which they studied with Lösch's "Amoeba coli," though it was really Grassi's "*Amoeba coli*," and not Lösch's organism. At first (1895*a*) they named their amoebae *Entamoeba coli*—the new generic name being proposed in ignorance of the similar name (*Endamoeba*) previously introduced by Leidy (1879). In their second publication (1897), however, they renamed the species completely, calling it *Entamoeba hominis*. Pestana (1917) has recently attempted to revive this name.

It may be noted that Casagrandi and Barbagallo (1897) introduced the name *E. hominis* in a most irregular manner. It occurs only twice in their publication—first as an implied synonym of "*Amoeba coli* (Lösch)" in the title of their paper, and a second time in the summary of their conclusions (p. 163), where it is again implied that it is synonymous with "*A. coli*." There can be little doubt that these authors actually studied the species here considered; but it seems probable that they also saw, and confused with it, other species. For example, they found individual amoebae as small as 5 μ in diameter, and these cannot have been *E. coli*. They also saw amoebae containing red blood corpuscles, and these must have been *E. histolytica*. They found cysts with diameters down to 8 μ, and these were, in all probability, those of *E. histolytica* or *E. nana*. They did not describe the nuclei in the cysts correctly—even as regards their number; for they stated that the cysts may contain "from 1 to 11 and more," although they figured some typical 8-nucleate specimens. Certain of their other observations will be considered later: for the present it will suffice to note that their "*Entamoeba coli*" or "*E. hominis*" was, for the most part, the organism here described under the first of these names.

Schaudinn (1903) restudied and redescribed this organism, adding numerous details—mostly wrong—to the earlier accounts. He considered that the amoebae studied by Quincke and Roos (1893) were all uncertain species, because "infections of cats" . . . "cannot be used as a specific criterion"*—which shows how superficial was his knowledge of the amoebae of man and of the work of these authors. Casagrandi and Barbagallo (1897), however, he allows to have studied his own "harmless form" from man with accuracy. Since they identified their amoeba with the "Amoeba coli" of Lösch, Schaudinn considered that this specific name (*coli*) should be used for the species which they described. He accepted, also, their generic name *Entamoeba* in place of *Amoeba:* and he concluded that the correct name of this organism is "*Entamoeba coli* (Lösch) emend. Schaudinn." This determination was not justified by the facts, as can easily be shown. Nevertheless, the organism has borne this name ever since.

Schaudinn's contributions to our knowledge of *Entamoeba coli* consisted chiefly in the discovery of a "schizogony" in the free amoebae and an "autogamy" in the cysts—both, in all probability, non-existent. He also confirmed the observations of earlier workers in regard to the structure and general appearance of the organism and its cysts, and as to its non-pathogenicity. The more important of his statements will

* This is, of course, true as a general statement. But in the present case it so happens that this criterion is a good one: and moreover it was by no means the only one used by Quincke and Roos.

be considered later. His only real discovery of any importance was that the mature cysts of this species are typically 8-nucleate : they do not contain the indefinite numbers of nuclei attributed to them by the earlier Italian workers.

For nearly a decade Schaudinn's work was generally accepted and "confirmed." The work of E. L. Walker (1911), followed by his later publication with Sellards (1913), then for the first time placed our knowledge of this organism on a sound scientific foundation. He proved conclusively that it is a distinct species, and by experimentally infecting human beings showed that it is non-pathogenic. From this date onwards little of importance has been discovered. Walker's work will be considered in greater detail later, but it is necessary to notice it here on account of its capital importance in the history of our knowledge of this species.

A few further words must now be said about the nomenclature of this amoeba. I have already discussed the subject very briefly elsewhere (1918), and have had to refer to it previously in the present work: but I cannot evade the question here. The present position is briefly this : "Amoeba coli" Lösch (1875), was not *Entamoeba coli* but *Entamoeba histolytica*—using these names to denote the species here described under them. "*Amoeba coli*" Grassi (1879 *a*) was, however, chiefly—if not entirely—*Entamoeba coli* : and so were *Entamoeba coli* Casagrandi et Barbagallo (1895 *a*), *Entamoeba hominis* Casagrandi et Barbagallo (1897), and *Entamoeba coli* Schaudinn (1903). The organism has been called *E. coli* by almost every worker since, and it is generally recognized by no other name. Now Schaudinn was unable to decide whether Lösch studied the form which he himself called *E. coli* or that which he called *E. histolytica*. His inability to do so can be explained by supposing that he had not studied Lösch's paper properly, or that he had not studied the amoebae of man properly—either or both of which may account for his singularly unfortunate pronouncements on the question : for there can be no doubt that Lösch's "Amoeba coli" was Schaudinn's *Entamoeba histolytica*, and not his *Entamoeba coli*. There is not a vestige of evidence that Lösch ever saw the latter species.* Consequently, Schaudinn was in error when he founded his species *E. coli* as a part of Lösch's species "Amoeba coli." As already noted in discussing *E. histolytica*, Schaudinn ought to have called this form *Entamoeba coli*—using the specific name first given to it. The first specific name available for the non-pathogenic species would then have been *hominis*—proposed by Casagrandi and Barbagallo (1897). His own "*Entamoeba coli*" would then naturally have been called *Entamoeba hominis*, and there would have been no further trouble. However, he made his mistake and nobody corrected him at the time. But it will create endless confusion if the name *Entamoeba coli* is now transferred to the dysentery amoeba ; and, as I have already stated, I should consider such a change—on the grounds of priority, or for any other

* As already noted on an earlier page, Mesnil (1918) believes that Lösch studied a mixture of species, and that the name *coli* can be given to one of these—the harmless one, as proposed by Schaudinn. M. Mesnil's judgement—for which I have the greatest respect—is so rarely at fault that I have, since reading his remarks, re-read the whole of Lösch's paper with the greatest care : but I am still unable to find the slightest evidence in support of his reading.

reason whatsoever—as contrary to the spirit, though probably conformable to the letter, of the Laws of Zoological Nomenclature. Accordingly, I accept Schaudinn's mistake and its consequences. I would point out, however, that the specific name *coli* was first given to the organism in question by Grassi in 1879—not by Lösch : and that the generic name *Entamoeba* was introduced, and combined with this specific name, by Casagrandi and Barbagallo in 1895. Consequently, if this amoeba continues to be known in future as *Entamoeba coli*, then its full title should be *Entamoeba coli* (Grassi, 1879) Casagrandi et Barbagallo, 1895 ; and not "*Entamoeba coli* Lösch emend. Schaudinn"—as Schaudinn would have it. It was not permissible to Schaudinn to amend Lösch's name so as to make it designate a different organism altogether : and if the name *Entamoeba coli* is retained in Schaudinn's sense, then the authorities cited for it should be the workers who first used it in this sense—namely, Casagrandi and Barbagallo, and not Lösch and Schaudinn.

The chief other synonyms of this organism are given in the list which heads this section. It only remains to add that *Entamoeba coli* is probably the amoeba that Schuberg (1893) found in non-dysenteric people ; the "*Amoeba coli*" of many earlier authors, and some later workers—*e.g.*, Massiutin (1889, Cases 2—5), Brumpt (1910) ; the "Amoeba I" of McCarrison (1909)—as he himself surmised ; and presumably the organism referred to as "Entamoeba Loeschi" by Lesage (1908). To this species also is referable the greater part of the "*Entamoeba coli communis*" of Knowles and Cole (1917), as Brug (1917 *a*) and Malins Smith (1918) have already pointed out. The 8-nucleate cyst of "*E. hartmanni*" described and figured by Prowazek (1912 *a*), the same author's "*E. williamsi*" (Prowazek, 1911), and the larger cysts of "*E. brasiliensis*" described by Aragão (1912, 1914), also all belong, in all probability, to this species. On the other hand, the "*Entamoeba coli*" of Werner (1912) probably comprises not only this species but also *E. histolytica*, *E. nana*, and *I. bütschlii*. Hartmann (1913, *et alibi*) considers that *Entamoeba minuta* Elmassian (1909) is a synonym of *E. coli* —which is obviously incorrect, as everybody who reads Elmassian's paper with any care must admit. Whether any of the organisms (and cells ?) called "*E. nipponica*" by Koidzumi (1909) were *E. coli* I am unable to determine with certainty. The *E. coli* of Akashi (1913) was chiefly this species, but apparently included *E. nana* as well. Brumpt (1913)—without stating his reasons—gives as synonyms of *E. coli* not only *E. hartmanni*, *E. williamsi*, *E. minuta*, *E. nipponica*, and *E. brasiliensis*, but also *E. bütschlii* Prowazek (1912 *a*), *E. polecki* Prowazek (1912), and the numerous "species" of amoebae cultivated from human stools by Celli and Fiocca (1894*a*). There is clearly no justification for the inclusion of these free-living forms. Fantham (1911) would include the free-living forms "*Entamoeba tropicalis*" Lesage (1908) and "*E. hominis*" Walker (1908) in *Entamoeba coli*—with which they have no connexion : and Calkins (1912) would include "*Amoeba lobospinosa*" Craig (1912), another free-living form. Craig (1917) even goes so far as to say that "*E. tropicalis* Le Sage" is certainly *E. coli*, though there is nothing in the original work of Lesage (1908) to substantiate this statement. From his description it was probably a mixture of free-living amoebae.

Whether any of the *Entamoebae* of monkeys are really identical with *E. coli* it is still impossible to determine (see p. 131), but the close similarity of *E. legeri* Mathis (1913) and the probably identical forms observed by

Wenyon (1908), Brumpt (1909), and others, should not be overlooked. Rodents—*e.g.*, mice—also harbour *Entamoebae* which are not at present distinguishable with certainty by any character, save their habitat, from *Entamoeba coli*. Brumpt (1910), indeed, formerly included the amoebae from mice, rats, guinea-pigs, and monkeys under *E. coli*—or, as he wrongly called it, "*Amoeba coli* Lösch." At present, however, this comprehensive conception of the species seems premature.

Description.

Entamoeba coli is one of the largest of the amoebae of man, though subject to great variation in size. Rounded individuals, belonging to the ordinary active stage of the organism, may have any diameter from about 18 μ to 40 μ. As a rule, however, in my experience, their diameter lies between 20 μ and 30 μ. As regards size, therefore, this species closely resembles *E. histolytica*. The living organisms, examined immediately after leaving the human body, are typically distinguishable from the latter species by the following characters:

They are far less active, as a rule merely showing changes of shape unaccompanied by active locomotion. Sluggish movements are characteristic of this species;* and the sudden extrusion of clear, blade-like pseudopodia—so often seen in *E. histolytica*—is never observable. Their ectoplasm is also far less differentiated, and the line of demarcation between it and the endoplasm usually not conspicuous. Their endoplasm is very bulky and granular, and usually heavily charged with food vacuoles containing various inclusions, but not red blood corpuscles or tissue elements. The nucleus is usually very conspicuous, appearing as a round or oval beaded ring, lying as a rule in an eccentric position. There is, of course, no contractile vacuole; but *E. coli* often contains vacuoles of a peculiar form,† resembling clefts or dilated cracks with pointed ends, and containing a liquid—possibly water. These vacuoles are quite different in appearance from the bubble-like vacuoles so commonly seen in degenerate *E. histolytica*. Whether, like these, they are formed as a result of degeneration I have not determined.

The food vacuoles may contain all manner of food derived from the contents of the bowel, and they give this species a very characteristic appearance in life. The structure of the cytoplasm is difficult to make out in consequence, but as a rule it appears coarser and more granular than that of *E. histolytica*. It often appears somewhat greenish in colour—probably owing to the contained granules. The colour, though slight, is quite distinct when individuals of the two species are seen lying side by side—*E. histolytica* appearing whitish and *E. coli* greenish.‡

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* Wenyon and O'Connor (1917) state that *E. coli* may sometimes show movements quite as active as those of *E. histolytica*. Though I do not question their observation, I may say that I have never been fortunate enough to see such lively specimens, and they must be very rare.

† These are shown in the amoeba depicted in fig. 55, Pl. IV.

‡ This description refers to the appearance of the amoebae under an ordinary achromatic lens. Under an apochromat, with good illumination, there is no appreciable difference in colour. The difference usually seen depends, therefore, in all probability, upon the difference in the granulation of their cytoplasms. It is purely an optical effect, and not due to the presence of any colouring matter.

Apart from the cytoplasm and its inclusions—which will be referred to again later—*E. coli* can be recognized most readily by its nucleus.

The nucleus of *E. coli*, like that of all other *Entamoebae*, contains most of its chromatin in a thin peripheral layer and a comparatively small karyosome. Its structure is shown in figs. 12 (Pl. I), 17 (Pl. II), and 55 (Pl. IV). It is typically round or slightly oval, and its diameter is usually from about 4 μ, in the smallest individuals, up to about 8 μ in the largest. In structure it is closely similar to the nucleus of *E. histolytica*, already described; but it differs in the following features. The external achromatic membrane is slightly thicker: the layer of chromatin within it consists of rather larger granules, more closely set together, though equally evenly disposed: the chromatic part of the karyosome is relatively somewhat larger—measuring about 1 μ in good-sized individuals: the cortex—or "halo"—of the karyosome is more definite and solid in appearance: the karyosome is nearly always eccentric—not central: and definite chromatin granules are generally present in the area between the karyosome and the peripheral chromatin layer. These distinguishing characters will be readily seen on comparing figs. 1 and 12 (Pl. I) and 16 and 17 (Pl. II). They are quite constant in perfectly fresh specimens which have been properly fixed and stained: but is is frequently impossible to distinguish *E. coli* from *E. histolytica* by its nuclear characters in poor preparations, or when the organisms are in the least degenerate.

Most of the published descriptions and figures of *E. coli* have been drawn, apparently, from a study of more or less degenerate amoebae. The large, discrete masses of chromatin so often shown at the periphery of the nucleus are only seen in degenerate organisms. The "ring" is really, in a normal animal, very uniform. The karyosome, to which too little attention has generally been paid, is rarely described in its normal form. It breaks up and disappears altogether in dying or dead organisms: and this accounts for the fact that in many figures it is depicted as an irregular mass of granules, or is conspicuous by its absence. Too little attention has also been paid to its position. In the nuclei of the vast majority of individuals it is not central, though often depicted in this position.* The presence in *E. coli* of chromatin granules in the zone between the karyosome and the peripheral layer is, in my experience, constant; and the importance of this character—as distinguishing the species from *E. histolytica*—has not been sufficiently emphasized, I think, because chromatin granules are so commonly seen in this position in the degenerate nuclei of the latter species. In *E. coli*, moreover, the peripheral chromatin layer is usually very resistant,† though the other constituents of the nucleus are not; and it may often be seen as a distinct ring of beads in an unfixed organism which has been dead even for days. Dead individuals of *E. histolytica* break up much more rapidly, and their nuclei disintegrate usually within an hour or two at most.

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* In a certain number of individuals the karyosome will appear to be central, of course, even if really eccentric—if the nucleus is viewed from the pole towards which it is displaced.

† And consequently, preparations of *E. coli*, showing fairly respectable nuclei, can often be made from stale material in which all the amoebae have long since died. It is unnecessary to emphasize the errors which may result from examining such preparations.

Food.—E. coli, as already noted, is a voracious and omnivorous feeder. In its food vacuoles may be found bacteria of all sorts, and every kind of vegetable débris from the contents of its host's colon (cf. fig. 17, Pl. II). Individuals may often be found containing starch grains and other plant remains. They also sometimes ingest the cysts of other protozoa such as lamblia (*Giardia intestinalis*),* coccidia (*Isospora hominis*),† and *E. histolytica*;‡ and they may even eat unencysted protozoa, according to some observers. Grassi, and Casagrandi and Barbagallo, found free trichomonads and *Giardia* within them, and O'Connor (1919) has confirmed the latter observation. In my cases I have seen only the cysts of this flagellate inside *E. coli*. §

Red blood corpuscles and tissue cells appear to be about the only things which *E. coli* will not eat. It is true that various observers—for example, Craig (1911)—note the occasional presence of such elements in the vacuoles of this species; but nobody has yet adduced sufficient evidence to place this observation beyond doubt. It is highly probable, indeed, that the individuals with ingested red corpuscles were really *E. histolytica* and not *E. coli*, the cases studied having been infected with both species. Mixed infections are extremely common; but *E. coli* containing red corpuscles—if they really do occur—are so uncommon that I have never encountered them. My observations agree in this respect entirely with those of Wenyon and O'Connor (1917). These workers also attempted to cause *E. coli* to ingest red corpuscles *in vitro*, but with negative results. There can be no doubt that it is sufficiently correct for all practical purposes to state that *E. coli* does not ingest red corpuscles; and that if an intestinal amoeba‖ is found containing these, then it belongs almost certainly not to this species, but, with the greatest probability, to *E. histolytica*. This fact is of the greatest service in diagnosis. It is also of interest biologically, for it marks the profound difference in food-habits between the two species—a difference clearly correlated with the great difference in their mode of life and pathogenicity.

It is now unnecessary to insist upon the fact that *E. coli* is a harmless commensal, possessed of no pathogenic powers whatever—so far as evidence is available: for only the inexperienced or prejudiced hold a contrary opinion. The early views of Cunningham, Grassi and Calandruccio, Casagrandi and Barbagallo, and many others, have, in this respect, been completely vindicated. It may be added that some of the Italian workers—especially Casagrandi and Barbagallo (1897)—have even gone so far as to maintain that *E. coli* is beneficial to its host; for it removes and destroys bacteria and waste matters of all sorts, and so acts as a useful scavenger of the large intestine.

Habitat.—All the evidence available goes to show that *E. coli* lives in the large intestine. The active forms live and multiply in the soft or

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* *Vide* Grassi (1888 *a*), Casagrandi and Barbagallo (1897), O'Connor (1919).

† *Vide* O'Connor (1919).

‡ *Vide* Wenyon and O'Connor (1917).

§ Capt. O'Connor has, however, shown me stained preparations of *E. coli* containing unencysted lamblia.

‖ The body in question must, of course, be really an amoeba. Endothelial cells in human stools—especially in cases of bacillary dysentery—are often mistaken by the inexperienced for *E. histolytica* when they happen to contain red corpuscles.

liquid contents of the upper part of the colon; and in the more solid and formed faeces in its lower parts, encystation occurs—the fully-developed cysts being normally discharged in the stools. This was first pointed out by Grassi (1882, 1883), and all subsequent work has shown that his conclusions were correct. At present there is no evidence that *E. coli* can live in any other part of the human body, or in any other manner—that it can invade the tissues, or give rise to secondary infections of the organs.

Multiplication.—There can be little doubt that *E. coli*, like other *Entamoebae*, reproduces by fission into two. This has been stated to occur by Grassi (1888) and many later workers; but nobody has yet described the process, and I doubt whether anybody has ever seen it. Schaudinn (1903) alleged that *E. coli* reproduces "by simple division," with an "amitotic" division of its nucleus: but he never described the process properly, and the rest of his observations on this organism are so full of errors that it is impossible to place much confidence in his mere statement. The earlier observations on "dividing" organisms, made by Casagrandi and Barbagallo (1897), are also very questionable. I think they merely saw binucleate amoebae—as many others, including myself, have done since—but not real division stages. Craig's (1911) figures of "multiplication by simple division in *E. coli*" (his fig. vi) I take to be diagrams drawn from imagination. Hartmann and Whitmore (1912) saw binucleate amoebae, and described "beginning stages" in nuclear division, with "heteropolar" and "tripolar karyosome-spindles." Hartmann (1912) has even asserted that 20-30 per cent. of all individuals of *E. coli* are found to be in early stages of division: but this is certainly incorrect, and a very fantastic interpretation of the appearances which he saw. In the "apparent division" stages of *E. coli* figured by James (1914), degeneration is, to me, far more apparent than division. Many other workers—for example Mathis and Mercier—refer to the division of *E. coli* as though it were well known to occur. Doubtless it does occur, but I can find no satisfactory evidence that anybody has yet observed it.

In stools containing large numbers of *E. coli* one can generally find individuals containing two nuclei, and sometimes nuclei which resemble the degenerate division stages occasionally seen in *E. histolytica* in human stools. Save for these questionable forms, I have never been able to find division stages of *E. coli*, in spite of much search: and consequently —although I have no doubt that it must occur, and often—I cannot give any description of the process of multiplication in this species.

Several workers have described a process of multiple fission or schizogony in *E. coli*. It was first suggested, I believe, by Casagrandi and Barbagallo (1897), who figured what they believed to be an 8-nucleate amoeba (their Pl. II, fig. 13): but they were unable to prove that multiplication occurs in this way. Schaudinn (1903) later alleged that *E. coli* undergoes multiple fission into 8 small amoebae—the process being preceded by a multiple division of the nucleus. No competent observer has ever confirmed this observation, although it has become a favourite with writers of text-books. It has been accepted by Craig (1911) and others, though the nearest approach to confirmation appears to be that of Akashi (1913): but judging from his figures, the "schizonts" are ordinary cysts of *E. coli*—showing various appearances commonly met with in preparations—and the brood of young amoebae formed by their

"schizogony" is apparently a suitably-posed group of 8 individuals of *E. nana*.

More recently, Mathis and Mercier (1917 *d*) have attempted to show that *E. coli* forms two kinds of cysts—"schizogonic" and "gamogonic." The latter will be considered later. In the former, they believe that the nucleus divides repeatedly until 8 to 16 are present in the cyst. The nuclear multiplication may take place partly in the amoeboid condition; and nuclei of variable size are often found, which are believed to reproduce by budding. The fully-formed cysts vary from 14 μ to 26 μ in diameter, but measure usually 17 μ. Their protoplasm may contain, even within the fully formed cysts, the remains of ingested food; and it is stated to be more alveolar, vacuolate, and eosinophile than that of the ordinary ("gamogonic") cysts. At the end of the period of nuclear multiplication, the protoplasm segments, the cyst bursts, and a brood of small amoebae is liberated. All this is said to occur in the same host, as a normal process of auto-infection.

I have never seen anything resembling this process, although I have studied a very large number of *E. coli* infections. I have, however, seen most of the forms which Mathis and Mercier interpret in this manner. Measurements of the cysts give no support whatever to their belief that there is a dimorphism of the type which they describe. Smith (1918) and Matthews (1919) have shown conclusively that it does not exist, and my own observations are entirely in agreement. The cysts with more than 8 nuclei, and with nuclei of unequal size, are, in my opinion, abnormalties. They do not occur in every infection, and similar nuclear abnormalities may be found in *E. histolytica*—a species in which they admit that no schizogony occurs. The "foreign bodies" in the "schizogony cysts" are merely small chromatoid bodies, such as so frequently occur in *E. coli* cysts—notwithstanding that Mathis and Mercier deny that they are ever found in this species. And their "amoebae" with more than one nucleus are merely, I believe, irregularly shaped cysts such as one often meets with in *E. coli* infections.*

One naturally attaches much importance to the statement of Mathis and Mercier that they have seen the emergence of the broods of small amoebae from their "schizogony cysts." On this point their words are unequivocal. Not only do they say that it is "not rare" to find this process taking place in fresh stools, but they also state that they have watched the living amoebae emerge. Their only figure† of the process, however, is highly unconvincing. The formation of the 8 young amoebae is not shown—merely a body, supposed to be a cyst of *E. coli*, with 4 small amoebae. Two of these are depicted within the cyst, and two without; and their nuclei are strikingly different from the nuclei which the authors figure in the earlier stages. Mathis (1913) had previously figured‡ a similar stage, which it is interesting to compare. One would be tempted to think that both figures were drawn from the

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* Casagrandi and Barbagallo were misled, I believe, in the same way by these cysts—as several other workers have also been since. Irregular cysts may easily be mistaken for amoebae in stained preparations, because—as shown elsewhere (Dobell and Jepps, 1918) for *E. histolytica*—the cyst wall becomes invisible when the cyst is mounted in balsam.

† *Vide* Mathis and Mercier (1917 *d*), fig. 15.

‡ *Vide* Mathis (1913), Pl. II, fig. 16.

same specimen, were it not for the fact that the position occupied by a nucleus in the earlier is occupied by an amoeba in the later figure. Except for this, and a slight difference in the position of the amoebae outside the cyst, the two cysts are strikingly alike.* They both contain a vacuole of a peculiar shape. So far as I am aware the presence of a large nucleus in the first cyst has not been explained; but it seems inconsistent with the later account of the process of schizogony. On such evidence, I am unable to believe in the "schizogony" of *E. coli*: and I believe that any worker who has studied this species with care, will, if he compares these figures with one another, and with the forms usually encountered in the stools, find little to convince him of the correctness of the interpretations of Mathis and Mercier. In spite of the most careful search—with many other workers—I have never succeeded in finding cysts of *E. coli* liberating broods of small amoebae; and the statement that this phenomenon is "not rare" is indubitably incorrect. It is so rare in fact that one may examine many hundreds of stools containing *E. coli*, and hundreds of thousands of cysts, without seeing any indication of it whatsoever. I regard the "schizogony" of Mathis and Mercier as an incorrect and arbitrary series of stages, and I am wholly unconvinced of the existence of this phenomenon in the life-history of *E. coli*.†

Encystation.—Before encystation *E. coli* undergoes a considerable reduction in size, forming precystic amoebae very closely similar to those of *E. histolytica*. These precystic amoebae (fig. 13, Pl. I) are very sluggish, or even motionless, entirely devoid of all food inclusions, and consequently colourless and hyaline in appearance. They usually measure from 15 μ to 18 μ in diameter. When alive their nuclei are rather more conspicuous than those of *E. histolytica* at the corresponding stage. The karyosome is slightly larger, as a rule, and more frequently eccentric; and the chromatin between it and the peripheral "ring" is rather more abundant. But all these differences are very slight, and not always observable. Since *E. histolytica*, as I have already noted, tends to assume similar characters at this stage, it is, in my opinion, frequently impossible to distinguish the precystic forms of the two species with certainty—either when alive, or in the best stained preparations. That these forms are often mistaken for one another I know only too well from experience. I have repeatedly been perplexed and mistaken in my attempts to determine them. If only these forms are present in a stool, it is, in practice, unwise to pronounce a definite diagnosis. The only safe course is to continue the examination of the stools until the ordinary vegetative forms—with their characteristic structure and cytoplasmic inclusions—or the cysts, make their appearance.

The precystic amoeba is doubtless formed—as in *E. histolytica*—by division of a large organism: but the dividing forms I have never been able to find. Its size also, as in this species, is proportionate to the size of cyst which it will produce.

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* The second figure of "schizogony" differs also in being inverted.

† Mercier and Mathis (1918) state that *E. ranarum*, a species apparently more closely related to *E. histolytica* than to *E. coli*, also forms cysts of two sorts—schizogonic and gametogonic. The latter are the cysts which I previously described in this species, whereas the former appear to be the amoeboid forms undergoing multiple fission originally discovered and described by Collin.

Encystation occurs in the same manner as in *E. histolytica*. In typical cases, the precystic amoeba becomes rounded and secretes its cyst wall, which also consists of a single layer, but is slightly thicker than that of *E. histolytica*. Inside the cyst the nucleus increases in size, and then divides into two. Each daughter nucleus again divides, forming four nuclei; and by a further division of each of these, 8 nuclei are finally formed. A glycogen vacuole appears in the cytoplasm at an early stage, and reaches its maximum development in the binucleate cyst. It is then absorbed, and the mature 8-nucleate cyst is typically without one. Chromatoid bodies may or may not be present at any stage in development. All these stages are shown in Pl. IV, figs. 56-62. After this brief summary of the development of the cyst, I may now consider several points in more detail, with special reference to the differences between *E. coli* and *E. histolytica*—since these are of great diagnostic importance.

Size of Cysts.—Cysts of *E. coli* may be found with all diameters from 10 μ up to 30 μ or even more. Cysts smaller than 10 μ have been described, but I have never seen them, and it is not unlikely that these very small cysts really belonged to a different species. They have all been described, at all events, by observers unacquainted with the smaller species living in man. Very large cysts—over 30 μ—are very uncommon, and are generally supernucleate abnormal forms. The largest I have ever found measured 33·5 μ, but Wenyon and O'Connor (1917) have recorded a still larger specimen, measuring 38 μ × 34 μ.

There can now be little doubt that *E. coli*, like *E. histolytica*, is a composite species consisting of a number of different races distinguishable by the sizes of the cysts which they produce. This was suggested by Wenyon and O'Connor (1917), and the measurements which I have made leave no doubts in my mind as to the correctness of their view. The question has recently been carefully studied by Matthews (1919), who has reached the conclusion that there are at least three, and probably four, distinct races of *E. coli*, with living cysts of the following mean sizes: 15 μ, 18·7 μ, 21·7 μ, and probably 16·5 μ. In all these races there is, of course, the usual variation in size around the modal value.

Malins Smith (1918) measured 1,000 cysts of *E. coli* from many different infections, and found their average size was 17·3 μ. This method, however, as I have already pointed out in considering *E. histolytica*, throws no light upon the existence of races. From Smith's figures, as Matthews (1919) points out, it seems probable that he studied at least several distinct races. That these races actually exist in *E. coli*, I think Matthews's figures show conclusively. He adopted similar methods to those which I used for *E. histolytica*, and his results are closely comparable. I believe, moreover, that the races of *E. coli* which he has demonstrated by no means exhaust the number actually occurring in this species.

The measurements of *E. coli* cysts published by Kuenen and Swellengrebel (1913) and Mathis and Mercier (1917 *b*) are too few to have any value in this connexion. Their curves—based in each case on measurements of only 100 cysts—are peculiar, but need not be further considered here. Their shortcomings have already been emphasized by Smith (1918) and Matthews (1919).

It has been proved beyond question that size is no certain criterion for distinguishing between the cysts of *E. coli* and *E. histolytica*. The sizes

of the cysts in these two species overlap to such an extent that only the extreme limits of size are of any diagnostic value. Cysts of both species may measure anything between 10 μ and 20 μ ; but a cyst measuring less than 10 μ is probably not a cyst of *E. coli*, and one measuring more than 20 μ probably does not belong to *E. histolytica*. Most English workers with a large experience are now in complete agreement on this point.*

Cyst Nuclei.—The resting nuclei within the cysts of *E. coli* have, at all stages of development, the same structure as the resting nuclei of the free amoebae. The eccentric position of the karyosome, the presence of chromatin granules on the linin network between it and the peripheral layer, and the other slight differences noted as observable between the nuclei of *E. coli* and *E. histolytica*, are constant at all stages. Far too little attention has previously been paid to this fact, most observers having emphasized the differences or resemblances in the number of the nuclei rather than in their structure. The structure is, however, of far greater diagnostic value than the number, in most cases ; for uninucleate, binucleate, and quadrinucleate stages in development are, of course, common to both species. The structural differences between the nuclei in the cysts of *E. coli* and *E. histolytica* will be readily appreciated by comparing figs. 3, 4, and 5 with fig. 14 (Pl. I), and figs. 62, 63, 66—69, with figs. 70—76 (Pl. IV). The position of the karyosome should be specially remarked. It is typically central in every nucleus in a cyst of *E. histolytica*, and eccentric in the nuclei of *E. coli*. The greatest carelessness has been shown by many workers in depicting the nuclei in the cysts of both species, but especially in those of *E. coli ;* of which there are many published figures showing central karyosomes in every nucleus in the cyst—though such appearances are practically never met with in healthy cysts with normal nuclei. In properly fixed and stained specimens it is usually possible to determine with certainty whether a given cyst belongs to one species or the other from the nuclear structure alone.

The size of the nuclei within the cysts of *E. coli* is also an important character. With successive nuclear divisions, the nuclei diminish in size, as in the case of *E. histolytica*. In typical, mature, 8-nucleate cysts, the nuclei are uniform in size; and they generally have a diameter which is between $\frac{1}{5}$ and $\frac{1}{6}$ of that of the entire cyst. (Cf. figs. 14 (Pl. I), 62, 68 (Pl. IV), etc.) The uninucleate cyst at the beginning of development possesses typically a nucleus which has approximately twice the diameter of each of the nuclei in the mature 8-nucleate cyst. The size relations are thus similar to those seen in *E. histolytica*. Consequently, in binucleate and quadrinucleate cysts of *E. coli* the nuclei are larger—relatively to the size of the cyst—than they are at the corresponding stages in *E. histolytica*. This will be clearly seen by comparing figs. 4 and 5 (Pl. I) or 71, 73, 74, 76 (Pl. IV) with figs. 59, 61, and 69 (Pl. IV). It is often possible to say with certainty whether a given 4-nucleate cyst belongs to *E. coli* or *E. histolytica* merely from the relative size of the nuclei (cf. figs. 69 and 71)—apart from the characteristic differences in their structure. Of the early stages in development of the cysts of *E. coli*, the 4-nucleate is that least often found in the stools. Binucleate and 8-nucleate stages

* Cf. Wenyon and O'Connor (1917), Dobell and Jepps (1917), Malins Smith (1918), etc. The measurements here given refer to living cysts, or those examined in iodine solution.

often occur in abundance, whilst the intermediate 4-nucleate stage may be rare or even absent. This is probably due to the fact that the cyst rests for a long time in the binucleate stage, and then rapidly completes the two further nuclear divisions. There is probably a very brief resting period at the 4-nucleate stage. This is also indicated by the fact that the majority of 4-nucleate cysts of *E. coli*, if carefully examined, show one or more of their nuclei preparing to divide or actually in the form of a spindle (cf. fig. 61, Pl. IV). This is often another very useful character for discriminating between the 4-nucleate stages of the two species.

It should be noted that the size of the nucleus in the uninucleate cyst of *E. coli* is very variable. At the beginning of development (fig. 56, Pl. IV) it is comparatively small, resembling the nucleus in the precystic amoeba (fig. 13, Pl. I). For the first nuclear division it then increases in size enormously (fig. 57, Pl. IV), finally forming a typical spindle (fig. 58, Pl. IV). The subsequent nuclear divisions are all similar, and resemble those of *E. histolytica*. Sometimes definite chromosomes appear to be present, however, as in the nuclear spindle shown in fig. 58 (Pl. IV): but I have not yet been able to satisfy myself of their invariable occurrence at all stages. I hope to return to this question later, when I have been able to examine more preparations. I would merely note here that, as in *E. histolytica*, the dividing nuclei in cysts discharged in the stools very soon become abnormal; and unless the cysts are fixed immediately, they show most misleading and abnormal nuclear figures. All the divisions appear to be accomplished in the same manner—the nuclear spindles, etc., in succeeding stages differing only in their size. Division of the nuclei is not always synchronous, and this occasionally leads to the production of cysts containing—probably only temporarily, in ordinary circumstances—odd numbers of nuclei, such as 3, 5, or 7. Cysts with 6 nuclei may also be found sometimes. Supernucleate cysts also occur from time to time. Instead of the nuclei entering into a permanent resting stage in the 8-nucleate cyst, one or more may again divide. In this manner cysts containing any number of nuclei from 9 to 16 may be formed. Fig. 67 (Pl. IV) shows a 16-nucleate cyst which is, as is generally the case, of unusually large size. Such cysts are not common, and do not represent normal stages in development, in my opinion.* They are, I think, supernucleate "freaks," like the 8-nucleate cysts of *E. nana* or the binucleate cysts of *I. bütschlii*, which are both equally rare. I have very rarely found cysts of *E. coli* containing more than 16 nuclei. I have seen cysts—examined in iodine solution—containing 18 nuclei, on several occasions; and I have once found one containing more—at least 20. The nuclei, which lie at different levels, are very difficult to count with certainty in such cysts, and the only safe way is to draw them very carefully with the camera lucida.†

Nuclei of different sizes are sometimes seen in the cysts of *E. coli*. I do not attach any importance to these, but regard them as abnormalities. They are, on the whole, uncommon: but occasionally an infected

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* Cf. Mathis and Mercier (1917 *d*), who regard them as "schizogonic" cysts.

† As an instance of the ease with which mistakes can be made, I may mention that I began to draw the cyst shown in fig. 67, Pl. IV in the belief that it contained 12 nuclei. It was only after every nucleus had been outlined with great care with the camera lucida that I realized my error.

person may pass a stool in which almost every cyst shows these atypical nuclear forms. They are probably the result of some change in environment caused by the diet of the host, or some similar factor.

The supernucleate and other abnormally nucleate cysts have already been noted by many workers. Casagrandi and Barbagallo (1897) were probably the first to notice these forms, and they appear to have believed that the number of nuclei in the mature cysts is inconstant. They seem also to have supposed that the nuclear multiplication within the cyst takes place by a peculiar process—a kind of endogenous budding. Schaudinn (1903) was really the first observer to emphasize the fact that the mature cyst is typically 8-nucleate. According to Smith (1918) some 87 per cent. of all *E. coli* cysts, encountered in random examinations of stools, contain this number of nuclei. It is thus an excellent specific character for purposes of diagnosis.

Schaudinn (1903), as is well known, described a remarkable process of "autogamy" during the development of the cyst of *E. coli*. There is not the slightest doubt that he was entirely mistaken in his interpretation—notwithstanding the "confirmations" which have been published by others. As Walker (1911) and Whitmore (1911) first pointed out, the development of the cysts of *E. coli* is perfectly straightforward and simple—just as I had previously described it in *E. ranarum*, and as is probably the case in all *Entamoebae*. The observations of Walker and Whitmore have been abundantly confirmed by every competent observer since, and all my own observations are in entire agreement with their interpretation.*

Glycogen.—E. coli typically contains more abundant glycogen in its cysts than *E. histolytica*, but it is present only in the earlier stages of development. The glycogen is often formed in the precystic amoebae, before the formation of the cyst wall. This can easily be demonstrated by treating them with iodine solution, when the glycogen shows itself in the form of one or more brown patches of variable size and stained with different degrees of intensity. In the uninucleate cyst the glycogen occupies an area of variable size. Sometimes it appears to be within the protoplasm, and sometimes between the encysted organism and its cyst wall. (Cf. fig. 56, Pl. IV.) As already noted, the glycogen is most abundant in binucleate cysts, in which it often occupies the greater part of the available room. In such cysts it appears as a solid mass, with a well-defined edge. It stains deeply in iodine solution, and gives the characteristic reaction with Best's specific carmine stain (fig. 15, Pl. I). In stained preparations which have been passed through water, the glycogen, is, of course, removed; and the area which it occupied is therefore seen as a vacuole (figs. 57-60, Pl. IV), usually with an irregular outline. A small amount of glycogen can often be demonstrated in 4-nucleate cysts of *E. coli*; but its presence in 8-nucleate cysts is extremely rare. It appears to be used up during development from the binucleate to the 8-nucleate stage. Cysts may occasionally be found devoid of glycogen in the early stages of development, but in my experience this is very rare.

* Although most other workers are very familiar with this fact, I emphasize the point here because Schaudinn's erroneous statements still unfortunately find acceptance with most writers of text-books—both zoological and medical.

The glycogen in the cysts of *E. coli* appears to have been first noted by McCarrison (1909); though he did not recognize it as such and merely recorded the presence of a "port-wine staining area" in the 2-nucleate and 4-nucleate, but not in the 8-nucleate, cysts of his "Amoeba I," when they were examined in iodine. His figures and account leave no doubt that he was dealing with the cysts of *E. coli*. Kuenen and Swellengrebel (1913, 1914) investigated the contents of the cysts more carefully, and showed for the first time that the iodine-staining substance is glycogen. Their observations have been repeatedly confirmed since. In my opinion the glycogen in the cysts of *Entamoebae* is a most important and useful diagnostic character (cf. Dobell and Jepps, 1917).

Several authors—including Wenyon and O'Connor (1917) and Mathis and Mercier (1917*d*)—regard the cysts of *E. coli* which contain glycogen as abnormal forms, incapable of further development. This view seems to have originated with Schaudinn (1903), but I think it is a mistake, and one due largely to the fact that too much importance has been attached to the appearances of the glycogen-containing cysts in stained preparations. In these, the area originally occupied by the glycogen appears as a clear space (cf. figs. 57-60, Pl. IV), or vacuole: and such "vacuolate" cysts may well appear to be degenerate if the fact that they once contained glycogen is overlooked. These workers, at all events, never even mention that the "vacuole" really contains glycogen—though this can be readily demonstrated. The "vacuole" is not an empty space, nor the result of degeneration. And when it is remembered that the majority of binucleate cysts of *E. coli* contain this glycogen "vacuole," and that similar glycogen inclusions occur in the cysts of most other parasitic amoebae at some stage in their development, it is difficult to believe that it is an abnormality. It is difficult to believe that most individuals of *E. coli* should store up glycogen—for no purpose—and then die without completing their development. That cysts containing a large glycogen "vacuole" are, moreover, capable of further development has been shown by Wenyon himself (1907) in the case of *E. muris*.*

Chromatoid Bodies.—As already noted, chromatoid bodies are not always present in the cysts of *E. coli*. They are often in the form of small granular or rod-like bodies in the earliest stages of development (fig. 56, Pl. IV), and are frequently larger and more abundant in binucleate cysts (fig. 60). In mature 8-nucleate cysts they are as a rule apparently absent: but well-stained preparations of freshly-passed cysts, if examined with care, generally show one or more very small chromatoid bodies lying among the nuclei (cf. figs. 14 (Pl. I), and 62 (Pl. IV)). Sometimes, however, the cysts of *E. coli* contain, at all stages of development, chromatoid bodies which are quite as abundant as those usually seen in the cysts of *E. histolytica*. They differ, as a rule, in form; being typically acicular or filamentar, and often aggregated into sheaves (figs. 63—66, Pl. IV). In general, they may be compared with fragments of splintered glass.

Sometimes the cysts of *E. coli* contain chromatoid bodies of a remarkable filamentar type (fig. 65, Pl. IV). The filaments are long and slender, and wound in a sort of skein inside the cyst, around and among the

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* He has observed and figured the "vacuole" at all stages in development of a living cyst of this species, though without noting the presence of glycogen in it.

nuclei. That these filaments are really chromatoid bodies, and not inclusions of some other sort, I think there can be no doubt: for stages intermediate (fig. 64) between the typical filamentar form (fig. 65) and the more usual acicular sheaves (fig. 63) are not difficult to find. These cysts with filamentar chromatoid bodies appear to be those which Prowazek (1911) described in his new species "*Entamoeba williamsi.*"* They were also, apparently, included in "*E. brasiliensis*" by Aragão (1912, 1914), and have been seen and figured by other workers.

According to Malins Smith (1918) chromatoid bodies occur in 5·5 per cent of the cysts of *E. coli*. This figure, however, is certainly too low; for it was arrived at by considering only those cysts which contain conspicuous chromatoids. All those with small chromatoid bodies, only demonstrable with certainty in carefully stained specimens, were left out of account.

Mathis and Mercier (1917 *a*, *b*, *et alibi*), apparently alone among recent workers, deny that chromatoid bodies ever occur in the cysts of *E. coli*. So far as I understand their views, they regard the deeply stained bodies in the cysts of this species as artifacts, formed by a deposition of stain (iron-haematoxylin) in folds of the cyst wall or in the protoplasm. It is easy to convince oneself that this interpretation is incorrect. The chromatoid bodies are easily visible in living cysts, and readily stained by carmine, haemalum, and other progressive methods. I can only suppose that these authors have devoted too little attention to the study of living cysts, and too much to iron-haematoxylin preparations. From their more recent papers (Mathis and Mercier, 1917 *e*, *f*) I gather that they now admit that chromatoid bodies are sometimes present in *E. coli* cysts, though they call them by a different name from that which they give to the chromatoids of *E. histolytica*.† This seems to me an attempt to fix an arbitrary verbal distinction where none exists in nature.

The chromatoid bodies of *E. coli* are probably formed in the same manner, and subserve the same functions, as those of *E. histolytica*. Their origin in the cysts is equally difficult to determine, and at present doubtful. I have never seen them in the precystic amoebae of this species, though they are possibly formed before encystation occasionally, as in *E. histolytica*. This is a very difficult point to determine, owing to the great similarity of the precystic amoebae of both species, and the frequency of mixed infections.

Chromatoid bodies were probably first noted in the cysts of *E. coli* by Grassi (1879). They were seen later by Casagrandi and Barbagallo (1897),‡ who regarded them as a sort of reserve material ("alimento

* Prowazek (1911, 1912) gives as a further character of this "species" the occurrence of cysts containing 10 or 14—15 nuclei. These, as already noted, sometimes occur in ordinary *E. coli* infections. It should be noted that Prowazek also stated that his "*E. williamsi*" occurred in company with *E. coli*.

† They now call the chromatoids of *E. histolytica* "bâtonnets sidérophiles," and those of *E. coli* "plages sidérophiles"; which clearly shows the undue importance which they give to the iron-haematoxylin method. Cf. Mathis and Mercier (1917 *e*). Chatton (1917, 1918 *a*) has already criticized their statements, and I have elsewhere pointed out the incorrectness of their observations in this respect (Dobell and Jepps, 1917).

‡ Their figure 18 shows, in my opinion, a cyst of *E. coli* containing a chromatoid body. Viereck (1907), however, regarded it as a cyst of *E. histolytica*—falling, I believe, into the very common error of attributing every cyst which contains chromatoid bodies to this species.

immagazzinato"). Schaudinn (1903) called them "chromidia," and since then they have generally been so named. With the "chromidium" of the shelled Rhizopods, however, they probably have nothing in common.

Mode of Infection.—What has been said already about the vitality and powers of resistance of *E. histolytica* cysts is equally applicable to *E. coli*, and need not be repeated. That the cysts are the forms which convey infection from man to man there can be no doubt. Grassi (1888) and Calandruccio (1890) state that they were able to infect human beings by causing them to swallow the cysts of this species. Schaudinn (1903) later asserted that he had twice* successfully performed this experiment upon himself. Carefully conducted experiments made later by Walker and Sellards (1913) have proved conclusively that infection is brought about in this manner. They fed 20 men on cysts of *E. coli*, belonging to 5 different strains (*i.e.*, from the stools of 5 different persons), and successfully infected 17 of them. Infection, as judged by the appearance of cysts in the stools, was established in from 1 to 11 days after feeding—the average time being 4·7 days.

The early stages in the development of the cyst in its new host are still unknown. On analogy with other species, it is probable that the cysts hatch in the small intestine, and liberate broods of 8 amoebae—or possibly a single 8-nucleate amoeba, which later divides into 8 young organisms—which pass into the large bowel, and there establish themselves.

Casagrandi and Barbagallo (1897) believed that they had been able to observe these stages in cats. They claimed to have seen the cysts hatch in the large intestine, and small amoebae emerge; but they believed that this development could only be observed in cats whose intestines had been previously irritated and inflamed by a special method of treatment. Their account is, to me, very unconvincing—as also is their figure, which resembles an artificially ruptured cyst,† but is supposed to show the emergence of the small amoebae. Schaudinn (1903) stated that he had confirmed these observations, and that "the results of my infection experiments on myself, and on experimental animals, agree completely with the findings of the two Italian investigators." No other workers, apparently, have ever been able to infect cats or any other animals with *E. coli*, or to observe any stages in their development in animals fed upon the cysts. Quincke and Roos, Darling, Wenyon, Craig, and many other workers always obtained negative

* This statement may well be questioned. Schaudinn stated that the infection lasted on each occasion for only 2 months, and then disappeared spontaneously. It is a remarkable observation, if true: for there can be no doubt that infections persist usually for many months at least, and even for years, and I have never seen a single case which has lost an infection with certainty whilst under observation. Apparent loss is very common—the stools of infected persons often remaining "negative" for long periods. Cf. Dobell (1917). Schaudinn's observations appear to me valueless, in the absence of any adequate control experiments. It is not improbable that he was infected with *E. coli* before, during, and after his experiments, but merely failed to find the cysts at certain examinations.

† Werner (1912) has figured a similar burst cyst—stained—which is supposed to illustrate the same stage; but, as he naively remarks, the bursting in this case was "the result of a trauma." It is by no means difficult to obtain such burst cysts—especially with certain fixatives; but it is difficult to see what connexion they can have with the development of the amoeba.

results. I do not believe it is possible to infect the cat with *E. coli*, or to obtain any stages of development in this host. In the experiments carried out with Dr. Dale in 1916 (*vide* Dale and Dobell, 1917) I studied a number of kittens which had ingested the cysts of this species, but I was unable to observe any development, and none of the kittens ever acquired an infection. I found, on several occasions, that some of the ingested cysts passed unchanged through the kitten's gut, and were discharged in its faeces ; but a larger proportion degenerated and died in transit, and I generally failed to find any forms—either cysts or amoebae—in the faeces of kittens which had ingested cysts of *E. coli*.

Conjugation.—At present there is no evidence of the existence of sexual phenomena of any sort in the life history of *E. coli*. The "autogamy" described in the cysts by Schaudinn (1903), and "confirmed" by several later workers, was certainly a mistaken interpretation—as already noted. "Conjugation" phenomena observed by other workers appear to be equally speculative, and are hardly worth even mentioning.

Mathis and Mercier (1917*b*, *d*) maintain that the cysts ("gamogonic cysts") of *E. coli* are of two sorts,—"macrocysts" and "microcysts," distinguishable by their dimensions. They are supposed to liberate macrogametes and microgametes respectively. The only concrete evidence advanced in support of their view is a remarkable curve—based upon measurements of only 100 cysts—incidentally introduced in one of their papers (1917 *b*). The much more extensive series of measurements of *E. coli* cysts made by Smith (1918) and Matthews (1919) have shown conclusively that Mathis and Mercier's conclusions are not justified. The dimorphism which they postulate in the cysts of *E. coli* does not exist. My own measurements also show clearly that there is no evidence of the existence of "microcysts" and "macrocysts."

Whilst there is thus no direct evidence of the existence of a sexual cycle in *E. coli*, it is by no means certain that conjugation does not occur. If it does, then the probability is that it takes place between the young amoebae recently issued from their cysts. To this extent I agree with Mathis and Mercier. I originally suggested a similar possibility in the development of *E. ranarum*, and Mercier (1910) soon afterwards stated that he had observed the whole sequence of events in *Endamoeba blattae*. His observations supply the only real grounds for supposing that a sexual cycle occurs in the development of any of the intestinal amoebae of man or other animals.

Cultivation.—*E. coli* has not been cultivated by any worker. It is certain, and will be conceded by everybody acquainted with the subject, that the "*E. coli*" cultivated by many of the earlier and some later investigators (*e.g.*, Fantham, 1911), was in reality some free-living species and not *Entamoeba coli*.

Occurrence.—There is now so much information regarding the occurrence of *E. coli*, and the facts are so generally recognized, that our knowledge of the distribution of this organism can be dismissed in a sentence. *E. coli* has been found living as a harmless commensal in the colon of man wherever and whenever it has been sought : no race, nor any country, has yet been discovered in which infections with this species are not common.

Treatment.—An *E. coli* infection cannot be eradicated by any known method of treatment. Emetine, various intestinal disinfectants, and other substances which have been tried are all inefficacious. There is a large

body of evidence on this question which it will be unnecessary to discuss here. See especially Wenyon and O'Connor (1917), Dobell (1916, 1917), Dobell, Gettings, Jepps, and Stephens (1918), etc.

(3) *ENTAMOEBA GINGIVALIS* (GROS, 1849) BRUMPT, 1913.

"Amoebea gengivalis" Gros, 1849.
Amiba buccalis Steinberg, 1862.
Amoeba dentalis Grassi, 1879.
Amoeba kartulisi Doflein, 1901.
Entamoeba buccalis Prowazek, 1904.
Entamoeba maxillaris Kartulis, 1906.
? *Amoeba pyogenes* Verdun & Bruyant, 1907.
Amoeba gingivalis (Gros) Brumpt, 1910.
Entamoeba kartulisi Doflein, 1911.
? *Entamoeba pulmonalis* Brumpt, 1913 (*nec* Artault, 1898).
Endameba buccalis Bass & Johns, 1915.
Endameba gingivalis (Gros) Smith & Barrett, 1915.
Endamoeba gingivalis (Gros) Smith & Barrett, 1915.
Endameba gengivalis (Gros) Lynch, 1915.
Endamoeba gingivalis (*buccalis*) Craig, 1916.
"*Endamoeba gingivalis* (Gros emend. Prowazek)" Craig, 1916.
? *Endamoeba confusa* Craig, 1916.
"Endamoeba Gros" Hecker, 1916.

History and Nomenclature.

The amoeba of the human mouth is of historic interest because it is probably the first parasitic amoeba discovered—not only in man, but in any animal. Like the dysentery amoeba, it was discovered in Russia. Its discoverer, Gros (1849), found it in the tartar on the internal surface of the teeth. His description is very brief, but his figures are recognizable. He named the organism "Amoebea gengivalis"—apparently intended for *Amoeba gingivalis*—and suggested that it might be spontaneously generated in the human mouth.

Some years later Steinberg (1862), also in Russia, found apparently the same organism, and named it *Amiba buccalis*.* Grassi (1879*a*) subsequently observed amoebae in the human mouth, and called them *Amoeba dentalis*, noting that they were possibly identical with Steinberg's forms. A few years afterwards, however, he expressed doubts as to whether the things which he studied really were amoebae or simply cells (Grassi, 1882, 1883).

Flexner (1892) and Kartulis (1893) found amoebae in the pus from maxillary abscesses. Kartulis found them in an abscess of the lower jaw of an Arab in Egypt. They were described and figured by him, but not named; though he threw out the suggestion that they might be the

* The original paper by Steinberg—sometimes cited as Sternberg—I have not been able to consult. A translation of the part dealing with the amoeba of the mouth is given by Smith and Barrett (1915), to whom I am indebted for my information concerning Steinberg's observations.

same as the "Amöba buccalis" of Steinberg and the amoebae of Grassi: and he also hinted that they might possibly be identical with "Amöba dysenterica," which they were said greatly to resemble. The amoebae of Kartulis were named "?*Amoeba kartulisi*" by Doflein (1901), *Entamoeba maxillaris* by Kartulis himself (1906), and later *Entamoeba kartulisi* by Doflein (1911). I agree with Smith and Barrett* (1915) that these names are probably synonyms of *E. gingivalis*.

Prowazek (1904) redescribed this amoeba, and renamed it "*Entamoeba buccalis* n. sp."—apparently in ignorance that it had already been observed and previously named *buccalis*. He was impressed by its resemblance to *E. coli*, and apparently considered it equally harmless.

Brumpt (1910) amended the name of the organism to *Amoeba gingivalis*, and later (1913) to *Entamoeba gingivalis* Gros, which appears to be the correct name of this species,—as Smith and Barrett (1915) have already pointed out† in their detailed analysis of the nomenclature. It is, moreover, the name now generally in use, though some recent workers still call the organism *E. buccalis*. Craig (1916), indeed, has used the curious combination "*Endamoeba gingivalis* (*buccalis*)," though in the same work he also names the amoeba "*Endamoeba gingivalis* Gros 1849, em. v. Prowazek 1904." Both these names are clearly incorrect: for the first is not in accordance with the rules of nomenclature, and the second attributes to Prowazek a name which he never used.

The amoebae described from an abscess in the malar region by Verdun and Bruyant (1907, 1907 *a*), and named by them *Amoeba pyogenes*, appears to me to have been *E. gingivalis*, though Smith and Barrett (1915) regard it as probably a distinct species. I also consider that *Endamoeba confusa* Craig (1916) is probably synonymous with *E. gingivalis*. It is a name proposed for an oral amoeba not yet properly described. From Craig's statements it appears to be closely similar to *E. gingivalis*, though said to be smaller. Its chief distinctive character seems to be "the liability of confusing this species with the smaller examples of *E. gingivalis*." At present there is no evidence to prove that such a confusion would be unwarranted. The name "Endamoeba Gros" employed by Hecker (1916) is evidently applied to *E. gingivalis*. I take it to be a term formed on analogy with the peculiar names used in bacteriological nomenclature.‡

The earlier workers made very few observations of any value upon *E. gingivalis*: and among the more recent workers there are but few who have supplied really accurate data from the zoological standpoint. Originally the organism excited but little interest. Indeed, it received no real notice until Smith and Barrett (1915) and Bass and Johns (1915) first§ advanced the hypothesis that *E. gingivalis* is the cause of pyorrhoea alveolaris (Riggs' Disease); Bass and Johns (1915) maintaining the

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* These authors unfortunately give both the names incorrectly, writing "*Endameba kartulisi* Döflein" and "*maxilaris*."

† They use the generic names *Endamoeba* or *Endameba*, however, on grounds of priority; though they presumably regard them both as interchangeable with *Entamoeba*. I do not follow Lynch (1915) in using Gros's (1849) original spelling of the specific name, which is clearly a misprint ("*gengivalis*" for *gingivalis*).

‡ Such as "Bacillus Flexner," "B. Shiga," etc.

§ Both these authors published preliminary papers in the preceding year (1914). Bass and Johns (1915) give the priority in the "discovery" to Smith and Barrett.

thesis that "the specific cause of pyorrhea dentalis and alveolaris is endamebas." All these workers went a step further and claimed that emetine has a specific therapeutic action in "oral entamoebiasis"—comparable with its action on *E. histolytica* in amoebic dysentery. These statements have been the subject of much controversy, and have been productive of a considerable amount of literature on the medical side. As they have now been to a great extent disproved, and as the controversy has little zoological interest, it will be unnecessary to consider it here in detail. The special points arising from it—in so far as they are concerned with the amoebae themselves—will be considered later.

On account of the supposed "specific relation" of the organism to disease, and the "specific action" of emetine upon it, some workers have even been led into discussing whether *E. gingivalis* is not really identical with *E. histolytica*. Smith and Barrett (1915), for instance, suggested this possibility; but later (1915*a*) they abandoned the hypothesis, and concluded that the two organisms are specifically distinct.* Although the characters which distinguish *E. gingivalis* from the intestinal amoebae of man appear at first sight somewhat indefinite, there can be no doubt now, I think, that it is an entirely independent species. Its resemblance to *E. coli*, noted by Prowazek (1904), or to *E. histolytica*, as stated by Smith and Barrett (1915), is not, I think, very striking to anybody familiar with all these species.

It may be added that the organism called "*Amoeba pulmonalis*" by Artault (1898) may possibly have been *E. gingivalis*: but I consider that the bodies which he saw were probably not amoebae but cells. Brumpt (1910, 1913), however, says he has also seen the organism, and gives some diagrams of it. He calls it *Entamoeba pulmonalis* (Brumpt, 1913), and says it may be identical with *E. gingivalis*. It seems to me not improbable that Brumpt's "*E. pulmonalis*" really was *E. gingivalis*, and was wrongly referred to Artault's species. Consequently, I have taken this view in enumerating the synonyms of *E. gingivalis* at the head of this section. It also seems not unlikely that the "ameba" found by Lynch (1915 *a*) in the lower jaw of an American negress suffering from "suppurative and hyperplastic osteoperiostitis," was really *E. gingivalis*. The author considers that it belonged to a new species, but as he has not studied or described the organisms properly—having accidentally destroyed his preparations—it is unnecessary to discuss this possibility. Since Kartulis and Flexner described "amoebae" from maxillary abscesses, many similar cases have been recorded, and at present there seems to be no sufficient reason to suppose that the organisms observed were not *E. gingivalis* in every case. Most of the descriptions are too meagre, however, to prove this conclusively: and I have little doubt that some at least of the "amoebae" found in suppurative conditions in and about the mouth have been endothelial or other cells, and not amoebae of any sort.

Craig (1916) records it as his opinion that "in all probability, further

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* These authors (1915 *a*) attempted to infect kittens with *E. gingivalis* by feeding them on pyorrhoeal pus rich in amoebae, and by injecting the amoebae into the rectum, or into the colon after laparotomy. They also injected amoebae *per rectum* into two puppies. All the experiments had negative results. They concluded that *E. gingivalis* is specifically distinct from *E. histolytica*, and that "intestinal infestment" is "probably impossible."

research will demonstrate that still other species of amoebae occur in the human mouth"; and he adds "it will be strange indeed if both *Endamoeba histolytica* and *Endamoeba coli*, the common intestinal endamoebae of man, are not sometimes encountered in this locality." This last statement appears to me to be a wild and wholly unwarranted prediction, which, if fulfilled, will indeed be strange. It will, indeed, be more—it will revolutionize our present conceptions not only of the amoebae but also of parasites generally. For the moment, however, there is no evidence whatever to support Craig's view, and there is very little in favour of the hypothesis that more than one species of amoeba inhabits the mouth of man.

Description.

Entamoeba gingivalis is a small amoeba which appears to show great variation in size. Prowazek (1904) gives its diameter as 6-32 μ; Chiavaro (1914) gives 5-20 μ; Mendel (1916) gives 5-40 μ; Goodey and Wellings (1916) give 7·5-27 μ; Goodrich and Mosely (1916) 10-30 μ; and Nowlin (1917 *a*) 12-40 μ; whilst Smith and Barrett (1915) state that the usual diameter is 30-35 μ, and that organisms may measure even more—up to 60 μ. In my experience this amoeba is usually much smaller, and its diameter is usually between 10 μ and 20 μ in fixed and stained specimens. I have not seen living organisms with a greater diameter than 25 μ, and as a rule they have been considerably less. My impression is that this species is typically smaller than *E. coli*, but I have studied only a limited amount of material.

The organism when alive resembles *E. coli* in general appearance, but is usually more active. Ectoplasm and endoplasm are fairly sharply differentiated, and the pseudopodia are well-developed, lobose, and typically rounded—not pointed. Individuals showing their pseudopodia extended are much more frequently seen in stained preparations of this species than in those containing *E. coli* or any of the other intestinal amoebae of man—all of which tend to become spherical when fixed. The most striking feature of the living amoeba is its cytoplasm, which is usually filled with numerous food vacuoles containing peculiar inclusions. In this respect it often resembles a well-fed small individual of *E. coli*. The nature of these inclusions will be considered later, but it should be noted here that they have a greenish, refractile appearance, and are roundish in shape, so that they are not unlike ingested red corpuscles seen with ill-adjusted illumination. Red corpuscles are, however, in my experience, invariably absent from the endoplasm.

The nucleus of *E. gingivalis* is typically spherical and vesicular, inconspicuous during life, and closely resembles those of *E. coli* and *E. histolytica* in structure. (See Pl. V, figs. 93, 94.) Its diameter in my stained specimens is generally from 2·5 μ to 3 μ, and it appears to be slightly smaller, relatively to the diameter of the organism, than the nucleus of *E. histolytica* or *E. coli*. The size is variously given by different observers as 2·5-4 μ (Goodey and Wellings, 1916), 2-5 μ (Smith and Barrett, 1915), or 3-6 μ (Goodrich and Moseley, 1916). There is probably a very thin achromatic membrane surrounding the whole nucleus, as in other *Entamoebae*. As a rule this is invisible, however, and the chromatin granules which are massed against its inner surface completely hide it. These granules are, in healthy and well-fixed

specimens, of uniform size and closely packed together, so that the nucleus appears as a very definite and even ring in optical section (figs. 93, 94). The ring is of approximately the same thickness as in *E. coli*, but is usually more uniform. The spherical karyosome (figs. 93, 94), which measures about 0·5-0·75 μ in diameter, is sometimes—but not always—surrounded by a pale area, like the "halo" in *E. coli* and *E. histolytica*. The karyosome itself appears to consist entirely of chromatin. According to Prowazek (1904) it consists of several separate granules, but I have not seen such a structure in normal individuals, and I believe his statement is incorrect. In position the karyosome may be either eccentric (fig. 93) or central (fig. 94). In this character the organism appears to occupy an intermediate position between *E. coli* and *E. histolytica;* its karyosome being not so constantly eccentric as in the former, nor so constantly central as in the latter. Between the karyosome and the peripheral "ring" of chromatin there is a clear space, which appears to me to be entirely free of chromatin or other granules in normal well-stained specimens. In this respect *E. gingivalis* thus resembles *E. histolytica*, and differs from *E. coli*.

Smith and Barrett (1915) state that a "centriole" is sometimes present in the karyosome. Prowazek also believed that there is a centriole—to judge from the statements of Hartmann (1913)—though according to his earlier interpretation he regarded the whole karyosome as a "nucleus" within the real nucleus (cf. Hartmann and Prowazek, 1907, p. 316). Smith and Barrett (1915, 1915 *a*) state that the nucleus itself is more often central in position than in *E. histolytica*. The exact position occupied by the nucleus of an amoeba is, however, difficult to define: and the "eccentric" nucleus of the *Entamoebae* is a character upon which too much reliance should not be placed.

The endoplasm of *E. gingivalis*, as already noted, usually contains numerous food-vacuoles which enclose peculiar ingested bodies. These are generally round or oval, and stain very intensely with nuclear stains; but if the stain is suitably extracted they are seen to be composed of granules of variable size. In specimens deeply stained with iron-haematoxylin they appear black and homogenous, and resemble the ingested red corpuscles in *E. histolytica*. That they are not red corpuscles, however, can easily be demonstrated by suitable staining and by examination of the living animal. From their staining reactions the bodies consist mainly of chromatin. According to Goodey and Wellings (1916) they are the "nuclei of degenerated and disrupted salivary corpuscles" occurring in the mouth. Goodrich and Moseley (1916) say that they are the "nuclei of lymphocytes or other mononuclear leucocytes." But Smith and Barrett (1915) appear to believe that the inclusions are partly leucocyte nuclei and partly red corpuscles. They believe that red corpuscles are frequently ingested by *E. gingivalis*; but they say that these are soon haemolysed after ingestion, so that they rapidly disappear. Goodey and Wellings (1916) deny that *E. gingivalis* ever ingests red corpuscles or polymorphonuclear leucocytes, but Goodrich and Moseley (1916) state that both of these "have been seen" within the organisms. Prowazek (1904) merely stated that the food consists partly of "leucocytes," but he did not specify the kind. Nowlin (1917 *a*) refers to the "solid masses" in the cytoplasm, but never observed *E. gingivalis* ingesting red corpuscles or leucocytes. This worker expresses the peculiar opinion that the organism "absorbs

its food mainly, taking in by osmosis the fluids of leucocytes or other media on which it rests." It is difficult to reconcile this view with the fact that the amoeba is frequently filled with what are obviously the remains of nuclei of some sort, and also with the same worker's earlier statement that the amoeba "can load up with a great cargo of cells and tartar." The most astonishing view, however, is that expressed by Craig (1916), who conjectures—for no apparent reason—that the inclusions in *E. gingivalis* are "some species of protozoan organism."

As I have already noted, I have never seen red corpuscles in *E. gingivalis*; but I am not prepared to deny that they are ever ingested by this species, in view of the statements of Smith and Barrett, Goodrich and Moseley, and others. That they are not ingested as frequently as the former authors believe, however, there seems good reason to suppose. Most of the inclusions are undoubtedly the remains of nuclei, either of salivary corpuscles or of other leucocytes or cells. I believe polymorphonuclear leucocytes are occasionally ingested. The organism figured in Pl. V, fig. 93, contains, I believe, the remains of one. Apart from these nuclear residues, *E. gingivalis* also ingests bacteria, which are usually present in large numbers in its vacuoles. These have been seen and described by Prowazek (1904), Smith and Barrett (1915), Goodey and Wellings (1916), and most other observers. Nowlin (1917 *a*) states that she has observed the living amoebae ingesting bacteria. I have not been able to watch this process myself, though it must occur frequently when the animal is in its normal environment.

E. gingivalis probably reproduces by division into two, but I have observed no stages in the process. Prowazek (1904) mentions simple fission as its chief mode of multiplication, and a figure of his, showing an amoeba with a "dividing nucleus," has been several times reproduced (cf. Hartmann and Prowazek (1907) p. 316, Hartmann (1913) p. 641, etc.) and interpreted in various ways. Originally it was said to show one nucleus dividing inside another; later, to be a single nucleus undergoing "promitosis." Stages in a process of mitosis are referred to by Chiavaro (1914), but not clearly described. A late stage in division is figured by Goodey and Wellings (1916), but they found no earlier stages. Goodrich and Moseley (1916) state that reproduction is effected by "binary fission," but the process does not appear to have been observed in detail. Nowlin (1917) records a "mitosis" in this organism, but gives highly unconvincing figures. She also believes that "budding" or "multiple fission" takes place, "merozoites" being formed to the number of "8 or 9 to more than a dozen." From the description and figures it appears probable that these were cellular elements of some sort from the mouth, and not amoebae. Other authors mention division, but no one has yet described it properly.

Cysts of *E. gingivalis* have been described by several workers. Smith and Barrett (1915) state that they "have found 'dauer' cysts, but thus far no reproductive cysts"—whatever that may mean. No descriptions are given. Craig (1916) describes both "cysts" and "precystic amoebae." The former are said to measure 8-10 μ in diameter, and to be uninucleate. "Larger cysts are sometimes observed," but all sizes are said to be very rare. According to Goodrich and Moseley (1916) Craig's cysts probably belonged to "limax" amoebae, which they believe to occur occasionally in the mouth. Craig truly says of the "cysts" previously found by Chiavaro (1914) that they are "far from convincing."

Nowlin (1917) also found "cysts" of *E. gingivalis*. Their size is not given, but they are said to be smaller than the active amoebae. "They usually show some faint, rounded inclusions, probably the remains of food vacuoles." (If this is correct, then they differ from all other amoebic cysts.) The nucleus is not mentioned, and the illustration of the "cyst" is very unconvincing. Mendel (1916) maintains a judicial reserve regarding the occurrence of cysts. I have never been able to find any cysts in the mouths of persons infected with *E. gingivalis*. Careful search for them has also been made by Smith and Barrett (1915), Goodey and Wellings (1916), and Goodrich and Moseley (1916). None of them succeeded in finding any, nor did Prowazek (1904). It seems to me very probable that this organism does not form cysts at all, but is disseminated in an unencysted condition by simple contact between mouth and mouth—as Goodrich and Moseley suggest. At all events, all the "cysts" hitherto found have been so imperfectly described—almost every important cystic character being left out of the descriptions—that it is impossible to accept any of them, on the evidence so far presented, as cysts of *E. gingivalis*.

Craig (1916) says he has observed in *E. gingivalis* a process "which I regard as conjugation." It is not described, however, and the observation is not confirmed by any other worker; and at present there is no evidence of the existence of any sexual development in this species. Prowazek (1904) had previously thrown out the equally unfounded suggestion that *E. gingivalis* "sporulates" in the same way as *E. histolytica*—as erroneously described by Schaudinn (1903).

Occurrence and Habitat.—E. gingivalis commonly occurs in the tartar of the teeth—where it was originally found by Gros (1849)—and also in the *materia alba* between and around them. It seems to be specially common in the *Leptothrix* masses on the inner surface. The organism is often abundant in the pus found in pyorrhoeal pockets, and in other oral suppurations. According to Smith, Middleton, and Barrett (1914) it also occurs in the crypts of the tonsils, and on the tongue according to Lynch (1915). I have once found it among the spirochaetes and fusiform bacteria in the throat of a patient with Vincent's angina. Probably the organism may occur in any part of the mouth, though it seems to be particularly abundant in suppurative conditions. Lynch (1915) found *E. gingivalis* on the healthy gums and false teeth of two old women who had lost all their own teeth, and who showed no signs of pyorrhoea or other dental disease.

Goodrich and Moseley (1916) have made the interesting observation that an amoeba "indistinguishable" from *E. gingivalis* occurs in pyorrhoeal pus from the mouths of dogs and cats. It thus appears probable that the organism is not confined to man.

Pathogenicity.—Bass and Johns (1914) and Smith and Barrett (1915) originally claimed that *E. gingivalis* is the cause of pyorrhoea. They found it almost invariably present in this condition, and constantly absent from healthy mouths. Their observations, however, have not been confirmed; and almost all the recent investigators who have devoted careful attention to the matter have concluded that *E. gingivalis* is probably a harmless organism.* Observations which have been pub-

* This is the opinion of Goodey and Wellings (1916), Goodrich and Moseley (1916), and many other workers. It was also the view of Prowazek (1904).

lished by Lynch (1915), Mendel (1916), Goodey and Wellings (1916), Goodrich and Moseley (1916), Williams, Sholly, Rosenberg, and Mann (1915), Mitchell, Culpepper, and Ayer (1916), and others, show conclusively that *E. gingivalis* occurs in normal healthy mouths. For example, Mitchell, Culpepper, and Ayer say: "it is evident that a very large per cent of normal mouths harbour" this organism. They found amoebae in the mouths of 21·6 per cent. of children with normal gums; and Mendel found them in 8 out of 36 children, and in 24 out of 42 adults, all of whom showed no evidence of pyorrhoea. It seems equally certain, on the other hand, that *E. gingivalis* occurs more abundantly in the mouths of persons with pyorrhoea and unhealthy gums. Thus, Mendel (1916) found the organism in 38 out of 40 persons with pyorrhoea; Williams, Sholly, Rosenberg, and Mann (1915) found them in 70 per cent. of children with "spongy, bleeding gums"; and Mitchell, Culpepper, and Ayer (1916) found them in 74·4 per cent. of children with "receding, spongy, bleeding gums." As *E. gingivalis* feeds largely upon bacteria and the nuclei of disintegrated cells in the saliva, it seems not improbable that a condition such as pyorrhoea—with abundance of bacteria and broken-down pus cells—is particularly favourable to their growth. This would easily account for the greater frequency of the amoebae in suppurative conditions.

E. gingivalis is by no means always present in pyorrhoeal pus, or in dental abscesses. I have examined at least one case of pyorrhoea, and the pus from two dental abscesses, with completely negative results after a very exhaustive search. Similar results have been recorded by others. The heaviest infection which I have seen was in a man with only slight pyorrhoea, but with a very dirty and ill-kept mouth. Scrapings from all parts of the teeth and gums showed large numbers of amoebae, often accompanied by *Trichomonas* and immense numbers of spirochaetes. I have also seen *E. gingivalis* on one occasion in a syphilitic lesion of the mouth—a mucous patch on the lower lip.

Hecker (1916) attempted to cause pyorrhoea by injecting *E. gingivalis* —washed by a special technique, to free the amoebae from bacteria— into the gums of a guinea-pig and a man. Repeated inoculations did not succeed in establishing an infection or in causing pyorrhoea. Prowazek (1904) found *E. gingivalis* in carious teeth, but the observations of Chiavaro (1914) and Mendel (1916 *a*) seem to show that dental caries is not caused by its presence, as some writers have suggested.

At present, therefore, there seems to be no good evidence to support the hypotheses that *E. gingivalis* attacks the tissues, that it is the cause of pyorrhoea, or that it is in any way pathogenic. The suggestion that the amoeba acts as a pathogenic agent by means of a "symbiotic relation" with certain bacteria, as suggested by Smith, Middleton, and Barrett (1914), seems equally unfounded. The organism appears rather to be a harmless commensal, like *E. coli*. Chiavaro (1914) considers that it is "most probably an adjuvant in the autodisinfection of the mouth;" whilst Goodey and Wellings (1916) suggest that, since *E. gingivalis* is a "scavenger" of bacteria, it "may therefore be considered as a useful rather than a harmful organism." The available evidence, however, appears to me to afford but little ground for regarding it as either harmful or beneficial.

Treatment.—As already noted, Bass and Johns (1914, 1915), Smith, Middleton, and Barrett (1914), Lynch (1915), and others, claimed to

have found that emetine has a specific action upon *E. gingivalis*, and they therefore advocated the administration of this drug in pyorrhoea. It will be sufficient to note here that these claims have never been substantiated, and that many workers have now found that emetine is not a specific cure for pyorrhoea or amoebic infections of the mouth. Goodrich and Moseley (1916), Mendel (1916), and others, have failed to observe any effects produced upon *E. gingivalis* by giving emetine to its host. Lynch (1915), who apparently believes in emetine as a specific for "oral endamebiasis," cites a number of cases in which it appears to have been useless. It thus seems very probable that emetine has no specific action upon *E. gingivalis*, and that the original claims were based upon insufficient evidence. No other substance has, up to the present, been regarded as a specific for infections with this amoeba.

VI.

GENUS *ENDOLIMAX* KUENEN & SWELLENGREBEL, 1917.

There is only one species belonging to this genus, namely:

ENDOLIMAX NANA (WENYON & O'CONNOR, 1917) BRUG, 1918.

? Entamoeba phagocytoides Gauducheau, 1908 (*pro parte*).
Chlamydophrys stercorea Elmassian, 1909 (*nec* Cienkowski, 1876).
? Entamoeba nipponica Koidzumi, 1909 (*pro parte*).
"Small amoeba" Wenyon, 1912.
? Vahlkampfia punctata (Dangeard) Chatton & Lalung-Bonnaire, 1912 (*pro parte*).
Entamoeba coli Werner, 1912 (*pro parte*).
? Vahlkampfia Whitmore, 1913.
Vahlkampfia Craig, 1913.
Entamoeba coli Akashi, 1913 (*pro parte*).
"Free-living amoebae" James, 1914 (*pro parte*).
Tetramitus mesnili Wenyon, 1915 (*pro parte*).
Amoeba limax Wenyon, 1916 (*nec* Dujardin, 1841).
Vahlkampfia Flu, 1916 (*pro parte*).
"Non-pathogenic *E. tetragena*" Shimura, 1916 (*pro parte*).
Entamoeba nana Wenyon & O'Connor, 1917.
"Limax" Swellengrebel & Mangkoe Winoto, 1917.
Entamoeba nana (Wenyon & O'Connor) Dobell & Jepps, 1917.
Endolimax intestinalis Kuenen & Swellengrebel, 1917.
Vahlkampfia nana (Wenyon & O'Connor) Brug, 1917.
"Limax amoeben" Flu, 1918.

History and Nomenclature.

Endolimax nana is so common an inhabitant of the human bowel that it is remarkable that it escaped recognition for so long. This was partly due, I believe, to its confusion with other amoebae—especially the small coprozoic and easily cultivable organisms commonly, but incorrectly, called "*Amoeba limax*."

Gauducheau (1908) described a small amoeba which he named *Entamoeba phagocytoides* and which he said lived "*in* the intestine of man"—a statement since reiterated many times. He believed he had been able to cultivate this species, but the forms in his cultures were clearly free-living amoebae. It seems possible, therefore, that he cultivated these from stools containing *E. nana*, and wrongly assumed the two organisms to be identical. His "*E. phagocytoides*" was probably, at all events, a mixture of different organisms: for it was found in fresh faeces, and isolated in cultures from stools, from liver abscess pus, and from water. It has been regarded by Gauducheau as having a genetic

connexion with *E. histolytica* and *Trichomonas*, and has had other remarkable and incredible characters attributed to it at different times.

Elmassian (1909) found some small amoebae in human stools and regarded them as naked forms of *Chlamydophrys stercorea*, which Schaudinn (1903) had stated to occur in human faeces. The figures (Elmassian, 1909, figs. 39, 40) apparently depict *E. nana*. They probably do not represent the rhizopod which Cienkowski (1876) called *Chlamydophrys*, and which I have never seen in human stools.

It seems to me probable that several of the Japanese workers have also seen *E. nana*, but misinterpreted it. The "merozoites" of "*Entamoeba nipponica*" described by Koidzumi (1909), the "young amoebae" formed by the "schizogony" of *E. coli*, described and figured by Akashi (1913), and similar forms of a "non-pathogenic tetragenous amoeba" figured by Shimura (1916, 1918), probably or possibly all depict *E. nana*. Wenyon (1912, 1913) undoubtedly saw *E. nana* and recognized it as a distinct species (cf. Dobell and Jepps, 1917). He figured a cyst later (Wenyon, 1915), but considered that it might belong to the flagellate *Chilomastix* ("*Tetramitus*") *mesnili*. He found the amoebae again in patients from Gallipoli (Wenyon, 1916), and called them "*Amoeba limax*"; but later, in a joint work (Wenyon and O'Connor, 1917), named the species *Entamoeba nana*.

As already pointed out elsewhere (Dobell and Jepps, 1917), the "free-living amoebae from the human intestine" described by James (1914), in Panama, were probably for the most part *E. nana*. This author states that he saw preparations of Wenyon's amoebae, and that they were the same as his own. This is probably correct; for I have also seen Wenyon's original preparations, and they certainly contain *E. nana*. But James says further that his amoebae were the same as those called "*Vahlkampfia punctata* Dangeard" by Chatton and Lalung-Bonnaire (1912). Now these authors believed that they had found a "limax" amoeba living in the intestine, and had succeeded in cultivating it. From their account it seems certain that they really did cultivate "*Amoeba punctata*" —which is *Dimastigamoeba gruberi* (Schardinger, 1899) Alexeieff, 1912*b*, a common free-living form which I have also cultivated from human faeces, soil, and water. It has been described under many other names. This organism, however, does not live in the intestine; and moreover it will not usually grow in cultures kept at the temperature of the human body. It appears highly probable, therefore, that Chatton and Lalung-Bonnaire were mistaken in supposing that the amoebae present in the intestine of their patient were the same as those in their cultures. What the intestinal forms really were it is impossible to tell from their account. They may have been *E. nana*, but they may also have been *I. bütschlii*. If James saw preparations of their cultivated form ("*A. punctata*" = *D. gruberi*), then he was also mistaken in supposing them to be identical with the forms which he himself had found (*E. nana*).

E. nana also appears to be the form that Craig (1913*b*) saw in James's preparations, and which he says was "a typical *Vahlkampfia*": and a similar form may have been seen by Whitmore (1913), who mentions and depicts a "vegetative form of *Vahlkampfia*" from a human stool. Flu (1916) also found a "Vahlkampfia" which was said to live partly free and partly in the intestine. Later (Flu, 1918) he appears to have come to the conclusion that this was *E. nana*; but he considers that this species is merely a harmless "limax amoeba adapted to a parasitic mode of life."

Werner (1912) apparently observed the cysts of *E. nana* but considered them to belong to *E. coli*. At all events, his crude figures (Pl. II, figs. 19, 20, 23, 29, 30) are far more like cysts of *E. nana* than those of *E. coli*, to which they are attributed.

E. nana was described as a separate species by Swellengrebel and Mangkoe Winoto (1917) under the peculiar name "limax": and later in the same year Kuenen and Swellengrebel (1917) described apparently the same form again, naming it *Endolimax intestinalis*. Brug (1917) pointed out that the organism described as *Entamoeba nana* by Wenyon and O'Connor (1917) could hardly be placed in the genus *Entamoeba*—as was, indeed, obvious: but his proposal to place it in the genus *Vahlkampfia* instead—as *Vahlkampfia nana*—was even more open to objection. No matter what interpretation is put upon this genus—and in my opinion the name *Vahlkampfia* should not be used for any organism—it is clear that *E. nana* has nothing whatever to do with any of the free-living amoebae for which it was intended. On the appearance of Kuenen and Swellengrebel's (1917) work, Brug (1918) corrected his earlier opinion; and as the work of Wenyon and O'Connor appeared before that of Kuenen and Swellengrebel, he pointed out that the specific name (*nana*) proposed by the former must stand, though the generic name (*Endolimax*) of the latter should be used instead of *Entamoeba* for the organism in question. Its correct name would thus be *Endolimax nana*.

This organism can hardly be placed in the genus *Entamoeba*, on account of the peculiarities of its nuclear structure and its cysts. On the other hand, there was—prior to the introduction of the name *Endolimax*—apparently no genus to receive it. Although I regard this generic name as inappropriate, in that it implies * a resemblance of this form to "*Amoeba limax*," I believe Brug's amendment must be accepted, as it is in accordance with the rules of nomenclature.

I would here call attention, however, to the apparent similarity of *E. nana* to a curious organism discovered by Minchin (1910) in the malpighian tubules of fleas (*Ceratophyllus fasciatus*), and named by him *Malpighiella refringens*. The systematic position of this parasite is still uncertain, though it is described as "amoeboid": and it forms 4-nucleate cysts with a structure apparently very closely similar to those of *E. nana*. Similar organisms have since been recorded by Alexeieff (1913) from the vagina of a leech (*Hirudo medicinalis*), and by Nöller (1914) from dog-fleas and rat-fleas. Neither of these authors, however, has ascertained definitely whether *Malpighiella* is an amoeba or not. The former thinks it is, the latter that it is not. If it really is an amoeba, then it seems not improbable that *E. nana* may have to be placed eventually in the genus *Malpighiella*, from which it seems at present to differ in no characters of generic magnitude. Unfortunately I have not yet been able to investigate *Malpighiella* with a view to deciding this question.

Attention may also be directed to an amoeba described from the faeces of frogs by Epstein and Ilovaiski (1914), and named by them *Naegleria ranarum*. The organism is said to be "semi-parasitic," and it certainly cannot be placed with propriety in the genus "*Naegleria*." The cysts of this species appear to be very like those of *E. nana* in certain

* This, at all events, appears to have been the authors' intention: though it may be remarked that *Limax* is the name proper to a slug, and therefore *Endolimax* would be a more suitable name for a parasitic mollusc than for a protozoon.

respects, and it is possible that the organism may eventually be found to belong to the same genus.

Description.

Endolimax nana has already been described more or less completely by Wenyon and O'Connor (1917), Swellengrebel and Mangkoe Winoto (1917), and Kuenen and Swellengrebel (1917). I have also given a brief account of it in an earlier joint paper (Dobell and Jepps, 1917), and shall therefore now merely note the points of chief importance.

E. nana is a small amoeba which usually measures, when rounded, from 6 μ to 12 μ in diameter. Living amoebae average about 8 μ, but fixed and stained specimens are generally about 1 μ less in diameter. The living forms somewhat resemble small free-living amoebae (so-called "*limax*" amoebae) at first sight, but this resemblance vanishes on closer study. They possess no contractile vacuoles, their nuclei are not clearly visible when alive, and their movements rapidly cease outside the body. Such movements as are usually observable under the microscope are like those of a small individual of *E. coli*—sluggish creeping, with few blunt pseudopodia showing a variable degree of distinctness between endoplasm and ectoplasm, soon followed by mere change of shape without locomotion, and ending in cessation of all movements. The cytoplasm usually contains many food-vacuoles filled with micro-organisms.

The most characteristic feature of the free amoeba is its nucleus, which can only be studied satisfactorily in well fixed and stained specimens from very freshly passed stools, and under high powers of the microscope. The nucleus is vesicular, and measures from about 1 μ to 3 μ in diameter, according to the size of the individual. As a rule its diameter lies between 2 μ and 2·5 μ. (See Pl. I, fig. 7, and Pl. II, figs. 18-23.) There is a peculiar karyosome in the nucleus of this organism, distinguished by the great diversity of form which it displays. It contains most of the nuclear chromatin and consists usually of one fairly distinct mass connected by threads or processes with one or more smaller masses. The main mass of the karyosome is usually eccentric in position, and consequently the organism often appears, when deeply stained or when seen under a comparatively low magnification, to contain a nucleus with a single rather small and eccentric karyosome. Figures of some of the commoner forms of nucleus are shown in Pl. I, fig. 7, and Pl. II, figs. 18-23; and fig. 24 (Pl. II) shows 8 other nuclei from other individuals. These drawings will convey a clearer idea of the nuclear peculiarities of this species than pages of description. It should be added that very careful study of the nuclei of these organisms—in really good preparations, and with the best lenses and critical illumination—shows that hardly any two individuals present precisely the same nuclear appearance. Several distinct types of nucleus can be distinguished—such as those represented by figs. 18, 19, 22, etc.—but there are many variants and intermediates, and the appearances differ, of course, according to the orientation of the nuclei in relation to the observer.

Apart from its karyosome the nucleus of *E. nana* presents no remarkable features. It possesses a well-marked nuclear membrane in which minute granules—possibly of chromatin—can sometimes be seen (figs. 18 21, etc.); and between the membrane and the karyosome there is

the usual "clear zone," sometimes traversed by radiating strands of "linin" (fig. 21). I have not been able to convince myself of the existence of any "peripheral chromatin" in the clear zone.

The nuclear appearances just described are those characteristic of this organism immediately after its discharge from the human body. If the amoebae are kept for some time subsequently, so that many of them degenerate and die, they show a considerable difference in nuclear structure. The karyosome segments run together into a more or less homogeneous mass, which then generally comes in contact with the nuclear membrane at one pole of the nucleus. The latter then has the appearance of a signet ring—the karyosome representing the signet. Such amoebae are certainly abnormal, as anybody can convince himself by examining a good series of stained preparations made from a stool containing large numbers of amoebae and fixed at different intervals of time after it was passed. It will then be found that it is only in the freshest samples that the true nuclear structure of *E. nana* can be seen.

The typically irregular forms of karyosome in this species were noted by Wenyon and O'Connor (1917). Swellengrebel and Mangkoe Winoto (1917) and Kuenen and Swellengrebel (1917), however, appear to regard a spherical karyosome as the normal form—apparently because they did not study sufficiently fresh material. Had they done so, they could hardly have considered—as they do, apparently—that *E. nana* is a kind of "*limax* amoeba." From all such organisms it is readily distinguishable by its karyosome alone. James (1914) shows an organism, which I believe to be *E. nana*, containing a nucleus with an irregular karyosome (see his fig. 117, Pl. xv). He probably saw the usual forms of the karyosome in this species, but mistook them for stages in division: for he says (James, 1914, p. 198) he saw "many organisms in various stages of division" in his preparations. But even in the freshest preparations division stages are excessively rare; and unfortunately he does not describe or figure his dividing forms.

Endolimax nana doubtless multiplies, like other amoebae, by division into two. I have not yet succeeded in finding more than a very few organisms which may show stages in nuclear division, and I am therefore unable to describe the process of fission at present. I would add that the various forms of karyosome encountered in individuals of this species appear to bear no relation to nuclear division: and although the different forms can be easily arranged in series with one another, there is at present no possible means of ascertaining whether such a series corresponds with a serial or cyclical change which takes place in the nucleus of one and the same organism during its life.

I have not succeeded in cultivating *E. nana* in any medium,—nor has any other worker, so far as I can ascertain. I may note, however, that I have cultivated free-living amoebae ("*limax* amoebae") from stools which originally contained *E. nana*, and I believe that other observers (such as Gauducheau, Noc, and Lesage) may have done the same: which may partly account for their belief that they had cultivated amoebae which normally live in man.

The habitat of *E. nana* is the human intestine, but the exact site of infection in the bowel is still in doubt. There are some reasons for believing that it may live in the small intestine (cf. Dobell and Jepps, 1917), but this has still to be verified. That it lives in the contents of the intestine, and is not a tissue parasite, is, however, certain.

I would note here that *Endolimax nana* is sometimes parasitized by a micro-organism belonging to the genus *Sphaerita* Dangeard. The infected amoebae, when alive, are very conspicuous objects, containing one or more spherical morulae of brightly refringent spores of the parasite. The spores are very symmetrically disposed, and at first sight resemble a mass of ingested micrococci enclosed in a food vacuole. Further study of such organisms, however, will show earlier stages in the development of the *Sphaerita* (cf. fig. 88, Pl. V), and discloses their true character. The spores stain deeply with iron-haematoxylin or haemalum (cf. figs. 87, 88), and on being carefully decolorized show very little internal structure (fig. 89). They measure about 0.75μ in diameter, and are consequently very difficult to study. Other stages are correspondingly minute, and I shall not describe them here. The parasite lives in the cytoplasm, and does not attack the nucleus (like the closely related form *Nucleophaga*), though the nuclei of many parasitized amoebae appear more or less degenerate. I have not seen *Sphaerita* within the cysts of *E. nana*—only in the free amoebae.

A closely similar form has been described in "*Amoeba limax*" by Chatton and Brodsky (1909), to whose work the reader is referred for further details (and literature) concerning these curious parasites of Protozoa. They have been described in several different free-living rhizopods and flagellates, but so far as I am aware have never previously been recorded in any parasitic amoebae. A related parasite—a *Nucleophaga*—has, however, been described in the nucleus of *Endamoeba blattae* by Mercier (1910).

I have now seen several *E. nana* infections in which a considerable proportion of the amoebae were infected with *Sphaerita*. The one which I was able to follow for the longest time was under observation for about three months, and I always found individuals parasitized by *Sphaerita* when free forms of *E. nana* were present in the stools. It thus appears probable that the infection is persistent. It seems, nevertheless, to have little effect upon the amoebae as a whole, for most of the *Sphaerita* infections which I have studied were in persons heavily infected with *E. nana*, who usually passed large numbers of normal cysts in their faeces.

I mention this parasite of *E. nana* here because of its interest and because the infected amoebae have—to my knowledge—already puzzled several people who have seen them. One worker who encountered them mistook the parasitized individuals for a new species of amoeba—the spore morula of *Sphaerita* being mistaken for a nucleus, whilst the nucleus of the host was overlooked. Other workers have mistaken *E. nana* individuals, with deeply-stained sporangia, for *Dientamoeba* and for cells containing masses of micrococci. *Sphaerita* has such a characteristic appearance in living amoebae that it cannot easily be overlooked, nor can its spores be taken for "nuclei" by a careful observer.

Cysts.—The cysts of *E. nana* are very characteristic structures, and contain, when mature, four nuclei. Precystic amoebae contain no food vacuoles, and their cytoplasm is consequently very clear. They are not distinctly smaller than ordinary active forms. Such individuals assume a rounded or oval form, and then secrete a thin cyst wall, which is colourless and perfectly smooth—as in most other intestinal amoebae. When newly formed, the cyst contains a single nucleus (fig. 25, Pl. II), and a

variable number of very small refractile granules. These are more clearly seen in the living cyst than in stained specimens. They give some of the metachromatic staining reactions of volutin, and are probably composed of this substance. They probably do not consist of chromatin, and are therefore not comparable with the chromatoid bodies in the cysts of *Entamoebae* and many free-living species. In uninucleate cysts the nucleus is usually relatively large (up to about 3 μ), and its karyosome relatively small, often having the form of a larger eccentric mass of chromatin united by a fine thread with a much smaller granule (cf. fig. 25, Pl. II)—a type of nucleus specially noted by Wenyon and O'Connor (1917). The nucleus later divides into two (fig. 26), and each of these again divides into two (fig. 27). Except for their progressive reduction in size, the resting nuclei in the uninucleate, binucleate, and quadrinucleate cysts show no change of structure. The arrangement of the chromatin in them is very hard to study accurately, on account of their very small size, but they show no conspicuous differences from the nuclear types observable in the active organisms.

Mature living cysts of *E. nana* are typically oval, and measure 8-10 μ in length and 7-8 μ in width. In fixed and stained specimens they appear slightly smaller. The nuclei in the mature cysts (stained) have a diameter of about 1 μ to 1·3 μ. (Cf. fig. 8, Pl. I, and figs. 27-29, Pl. II.) Except for the volutin granules already noted, the mature cysts typically contain no visible contents besides their four characteristic nuclei. The latter often lie near together towards one end of the cyst, but they may occupy any positions in relation to one another. In iodine solution the cysts stain a uniform yellow colour, and their nuclei are, as a rule, inconspicuous; though their karyosomes can generally be made out, with a little care, in this medium, and sometimes their nuclear membranes as well.

If a stool containing a large number of encysting amoebae and newly-formed cysts is examined in iodine solution, it will generally be found that many of the former show diffusely stained brown patches in their cytoplasm: and among the encysted forms there is usually but little difficulty in finding some which show definite contained masses giving a typical glycogen reaction. These glycogen masses are usually particularly prominent in binucleate cysts—as in *E. coli;* but they are also found sometimes in quadrinucleate and uninucleate cysts. They give other characteristic reactions of this substance, and show typical staining with Best's specific carmine method (see fig. 9, Pl. I). Glycogen cannot always be demonstrated in the cysts of *E. nana*, and it is usually commoner in the cysts contained in soft and diarrhoeic stools—in which free and encysting forms and young cysts are numerous—than in those found in formed and solid stools—which contain a large proportion of mature cysts and no free amoebae. Mature cysts of *E. nana* remain unchanged in human faeces for several weeks, if they are not desiccated. But the glycogen, if present originally in them, disappears completely— as noted already by Swellengrebel and Mangkoe Winoto (1917).

Several variations in the cysts of *E. nana* require further notice. Their form may range from the typical oval to that of a sphere. Very rarely they are of irregular shape, showing slight constrictions or bulgings which give rise to a variety of different forms. Their size, too, varies. Very small cysts, down to 6 μ in mean diameter, and very large ones, up to 11 μ or slightly more, may occasionally be met with (cf. figs. 30 and

31, Pl. II). And it is possible—and perhaps probable—that there are different strains of this species which can, as in *E. histolytica* and *E. coli*, be distinguished by the dimensions of their cysts.

As a rule the mature cysts of *E. nana* contain only four nuclei. But cysts containing eight nuclei are to be found (fig. 86, Pl. V), though these are very rare. Up to the present I have not seen more than a dozen such cysts among very many thousands examined. Swellengrebel and Mangkoe Winoto (1917) say that they have seen cysts containing five and six nuclei, but I cannot confirm their observation, and from their figures I doubt its accuracy. Cysts showing at first sight more than four nuclei are often met with : but careful study of these shows generally that the supernumerary "nuclei" are really deeply-stained "volutin" granules, or that a single nucleus with a bipartite karyosome has been counted as two nuclei. The same authors hint at a possible "autogamy" within the cysts of *E. nana*, and at other nuclear phenomena. It is certain, however, that such suggestions are unjustifiable, and that development in the straightforward manner described above is the rule. In all the thousands upon thousands of cysts of this organism that I have examined from hundreds of infections, there is not the slightest indication of any nuclear phenomena beyond those which I have described.

Occasionally the cysts of *E. nana* contain peculiar inclusions resembling rods or granules. They are sometimes long and filamentar, and sometimes in the form of definite bundles of short rods or heaps of coccus-like granules. Two cysts with such inclusions are shown in figs. 28 and 29, Pl. II. Those with filaments in them can be mistaken for small cysts of lamblia (*Giardia intestinalis*). The inclusions are visible in the living cysts, and are well seen in those stained with iron-haematoxylin ; but they are not easily distinguishable in cysts stained by most other methods. Such cysts appear to be characteristic of the infections in certain individuals. At all events, I have found that when they are present in the stool of a given individual on one occasion, they can be found again in his stools subsequently for a period of at least several months. What these inclusions are I am unable to state. From their forms one might suppose that they are parasitic or symbiotic bacteria ; but they may possibly be structures comparable with the chromatoid bodies of *E. histolytica* and *E. coli*.

Occurrence.—*E. nana* is one of the commonest inhabitants of the human bowel. Since the appearance of the work of Wenyon and O'Connor it has been found by every competent worker who has made a protozoological study of human stools. It occurs in the faeces of persons who have never left the British Isles, and is plentiful among British troops invalided to England from the tropics and from France. There are now numerous records available showing its frequency (see, for example, Wenyon and O'Connor (1917), Dobell and Jepps (1917), Mackinnon (1918), etc.). The cysts of this organism are small, and easily overlooked. Consequently, the incidence of infection is higher than would appear from most published records. A series of British soldiers—156 cases, consisting entirely of dysenterics from abroad, and all infected with *E. histolytica*—which I examined (in conjunction with Miss Jepps) with great care, in order to determine the exact incidence of infection with this organism, showed that it was present in no less than 33·3 per cent. Every case was examined at least seven times, and many

of them oftener. (*Vide* Dobell, Gettings, Jepps, and Stephens, 1918.) Other series have shown comparable figures,—allowance being made for the degree of thoroughness with which the examinations have been carried out. There can thus be no doubt that *E. nana* is of frequent occurrence in man.

Pathogenicity.—Although most of the infections with *E. nana* hitherto recorded have been found in persons who have had intestinal ailments, there is no reason to suppose that the organism is pathogenic. Wenyon and O'Connor (1917) found it in the stools of healthy men, with no history of dysentery or bowel trouble, and I can confirm their observations in this respect. From such figures as are available I can find no appreciable difference in the frequency of infection with this amoeba in healthy people and in those with intestinal disorders. Moreover, *E. nana* appears, in its habits of life, to resemble *E. coli*. It feeds chiefly upon bacteria in the contents of the gut, and there is no evidence that it can injure the tissues. It seems to me certain, therefore, that *E. nana* is a harmless commensal, and not a pathogenic parasite.

Treatment.—I may add here that no treatment has yet been found which will remove an infection with *E. nana*. Emetine administered to an infected individual—either hypodermically or *per os*—never removes the organism permanently, though it may disappear temporarily from the stools during treatment. I have now studied a large number of cases who have received emetine treatment, and have seen no exception to the general rule. No other substances—such as various intestinal antiseptics, etc.—which have hitherto been tried, have any action upon the organism in the human body.

VII.

GENUS *IODAMOEBA* NOV. GEN.

Up to the present only a single species belonging to this new genus is known. This is the form which I shall call:

IODAMOEBA BÜTSCHLII PROWAZEK, 1912 (*EMEND.*).

Entamoeba bütschlii Prowazek, 1912.
? *Entamoeba tetragena* Hartmann, 1912 (*pro parte*).
? *Entamoeba coli* Werner, 1912 (*pro parte*).
? *Vahlkampfia* sp. Chatton & Lalung-Bonnaire, 1912 (*pro parte*).
? "Free-living amoebae" James, 1914 (*pro parte*).
"Spherical bodies," Wenyon, 1915.
"Iodine cysts" or "I. cysts," Wenyon, 1916.
"I. cysts," Wenyon & O'Connor, 1917.
"Joodcysten," Brug, 1917.
"Pseudolimax," Kuenen & Swellengrebel, 1917.
Entamoeba tetragena Flu, 1918 (*pro parte*).
Endolimax Williamsi Brug, 1919 (*nec* Prowazek, 1911).

History and Nomenclature.

The first recognizable account of this amoeba seems to me to be that of Prowazek (1912 *a*), who named it *Entamoeba bütschlii*—at the same time noting that "the designation *Entamoeba* is provisional, since we do not know the life-cycle." His description is very imperfect. He saw but a single infection, in a child from the Caroline Islands in Saipan (Ladrones). The case was also infected with *E. coli* and other organisms.

Prowazek states that his amoebae measure 10-24 μ (presumably in diameter, when rounded), and that their nuclei are vesicular, with a round central karyosome containing a centriole. Between the karyosome and the membrane there is a network with chromatin granules distributed on it. "Cyclical processes" are observable around the karyosome, and stages in "nuclear division" are described. "Division" stages with two and three nuclei are figured. The cyst is also figured, but not described. It is merely said to be round, with a distinct membrane, and entirely different from that of *E. coli*. The specimen figured is said to have measured 14·8 μ. It is uninucleate, with its protoplasm apparently shrivelled.

Hartmann (1912) has figured an organism which he calls a "young amoeba" of *Entamoeba tetragena* (his fig. 2 *a*, *b*), and two other organisms—described as "degenerate forms" of the same species (his figs. 15, 16)—which appear to me to be probably the form under discussion. They certainly do not look like *E. histolytica*, at all events.

In the same publication Werner (1912) figures three structures which he calls "uninucleate cysts of *E. coli* with vacuoles" (see his figs. 16, 17, 22) : and these also appear to me to have been drawn from the present species. But the figures are extremely bad, and at all events quite unlike the cysts of *E. coli*.

It seems to me possible that the "free-living amoebae" which Chatton and Lalung-Bonnaire (1912) found in the human intestine, and identified as *Vahlkampfia punctata* Dangeard, were really *I. bütschlii*. As noted already—in considering *E. nana*—it is highly probable that they were not "*V. punctata*," which these workers cultivated from the same stool. Their description of the intestinal forms is too meagre, however, for me to identify them with certainty ; but to judge from the figures they were certainly not unlike the form under consideration. The same remarks are applicable to some of the "free-living amoebae from the human intestinal tract" noticed by James (1914) in Panama. His figures 114-116 are certainly suggestive of *I. bütschlii* rather than *E. nana*, to which species the rest of his "limax" amoebae should probably be referred.

Wenyon (1915 *a*) briefly described and figured some "spherical bodies," containing an "iodophilic" inclusion, found in human faeces. Somewhat later (Wenyon, 1916) he redescribed them under the name of "Iodine cysts" or "I. cysts." A fuller account, with some further facts concerning them, was published by Wenyon and O'Connor (1917), who gave them the same name. Wenyon (1915 *a*) regarded these bodies as "probably of a vegetable* nature," and stated later (1916) that this "is proved by the fact that they germinate when kept in faeces." Dr. Wenyon called my attention to these bodies in 1915, and since then I have studied them in stools from many different persons. At first I agreed with him that they were probably vegetable organisms, but I now know that they are really the cysts of the amoeba described in this section. They have become familiar—since Wenyon's account—to most workers in England under the name of "I. cysts," or "I. bodies." Brug (1917), in Java, redescribed them as "Joodcysten," and regarded them as probably "a new sort of parasite" altogether—what sort, he did not suggest.

Later, Kuenen and Swellengrebel (1917) found both the "I. cysts" and the amoebae which form them in a single case, and provisionally named the organism "Pseudolimax"—a name which they fortunately state to be not subject to the laws of nomenclature. They made no reference to the work of others on the same form, and apparently regarded it as a kind of "limax amoeba"—which it certainly is not. Brug (1918) subsequently pointed out that their "pseudolimax" was the organism of the "I. cyst." Another Dutch worker, Flu (1918), has since found the "I. cysts" once more, and has concluded that they are degenerate cysts of *E. histolytica*—or, as he terms it, "*E. tetragena*." This is undoubtedly incorrect.

I first saw the living amoebae† which form the "I. cysts," I believe,

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* Matthews (1918) also states that "they probably represent some stage in the life-history of a vegetable organism."

† I may say that all my own work on this organism has been done in entire ignorance of the simultaneous investigations of the Dutch workers, whose papers I was not acquainted with until my own work was completed.

in a preparation which Col. Wenyon showed me in the summer of 1917. At that time, however, their connexion with the "I. cysts" was uncertain, though it was one of several possibilities which we discussed. So far as I am aware, Col. Wenyon did not pursue the investigation of these forms further. I continued to look for the amoebae again, however, in other cases; and I saw them once more in another case which I studied at the end of 1917, and in yet another at the beginning of 1918: but I was unable to prove conclusively that the "I. cysts" and amoebae were genetically connected, and on both occasions the amoebae were mostly dead and degenerate. During 1918 I studied several further cases of infection, but with little better success until the autumn. When my assistant, Miss M. W. Jepps, who had learnt of the "I. cysts" and amoebae from me, left me in the spring, and went to Southampton, I suggested that she should continue to study cases of "I. cyst infection" with a view to clearing up the matter. This she did, and in the late autumn I received from her a preparation containing numerous "I. cysts" and amoebae, which she had observed alive, from a case which she was studying. A few days later I was fortunately able to study the living amoebae, the cysts, and all intermediate stages, in great detail, in another case which Major G. C. Low kindly allowed me to examine. After a very careful study of Miss Jepps's preparation and those which I had myself made from Major Low's case, I felt convinced that the "I. cysts" and the peculiar amoebae are stages in the development of the same organism. Since then I have encountered the amoebae and cysts again in other cases, and Capt. F. W. O'Connor has very kindly allowed me to study further specimens containing both amoebae and cysts of this species from cases which he investigated in Egypt in 1917. I have now no doubts as to the general correctness of the description given below. There is, I think, no reason to doubt that the "I. cyst"—in spite of its remarkable structure—is really the cyst of an amoeba; and that this amoeba is, moreover, the organism which Prowazek (1912*a*) imperfectly described, and named *Entamoeba bütschlii*.

Brug (1919), in a paper just published, has returned to the study of this organism and identified it with Prowazek's "*Entamoeba Williamsi*" —which was really, as has already been pointed out, *E. coli*. Brug considers that the organism should be referred to the genus *Endolimax*. The type of this genus is, however, *E. nana*—an organism whose cysts and nuclear structure are entirely different. His conclusion that the "I. cysts are the cysts of Entamoeba Williamsi, Prowasek (*sic*). The latter should be called: Endolimax Williamsi", is one in which no protozoologist with a systematic knowledge of the amoebae can possibly, I think, concur.

It is clear that this organism cannot be placed in the genus *Entamoeba*, on account of its nuclear structure and the characters of its cysts. Nor is there any other genus of amoebae in which it can be correctly placed; and it thus seems necessary to create a new one to receive it. The name "Pseudolimax," given by Kuenen and Swellengrebel (1917), was not proposed in accordance with the rules of nomenclature as a generic name, and is also inappropriate. As I think it desirable to preserve the historic connexion between the amoeba and Wenyon's "Iodine cysts," which are already so well known to many workers, I therefore propose the generic name given above—*Iodamoeba*—for this organism. Its name, accordingly, becomes *Iodamoeba bütschlii* Prowazek, 1912.

It may be added that Brumpt (1913), James (1914), and Pestana (1917), have considered Prowazek's "*Entamoeba*" *bütschlii* to be *E. coli*. James, indeed, says that its "cysts can in no way be differentiated" from those of the latter. This I conceive to be a quite unjustifiable interpretation. Kuenen and Swellengrebel (1913) suggested that "*E.*" *bütschlii* is a degenerate "*minuta*" (*i.e.*, precystic) form of *E. histolytica*, and Flu (1918) seems to consider it also as a degenerate form of this species. This also I regard as very wide of the mark. I believe that none of these authors had sufficient material at their disposal to enable them to form a correct opinion regarding this organism. Wenyon and O'Connor (1917) have called attention to the resemblance of Prowazek's "*E.*" *bütschlii* to *E. nana*, but note that "this author's description is too meagre to allow of any comparison being made"; and they add that "he does not describe any encysted forms"—which is not quite correct, though it might perhaps be asked how far Prowazek's rough account can properly be termed a "description."

Description.

Iodamoeba bütschlii is, as a general rule, a small amoeba intermediate in size between *E. coli* and *E. nana*. I believe I have, in the past, sometimes mistaken the living organisms for small individuals of the former species or large ones of the latter. The diameter of the living amoebae, when rounded, is usually about 9—13 μ; but larger individuals up to 17—20 μ, and very tiny ones down to 5 μ, are also found. I have never seen any as large as 24 μ—the maximum size mentioned by Prowazek (1912 *a*). Since he studied a case infected with *E. coli* also, it is possible that he mistook some individuals of this species for *I. bütschlii*. According to Kuenen and Swellengrebel (1917) the amoebae measure 10—12 μ in diameter: according to Brug (1919) 12—20 μ.

In general form and habit this organism is closely similar, when alive, to a small specimen of *E. coli*. Ectoplasm and endoplasm, pseudopodia, and the sluggish movements when outside the body, are closely alike in the two species. The cytoplasm of *I. bütschlii* is also frequently filled with food-vacuoles containing numerous bacteria and other foreign particles; and a contractile vacuole is, of course, likewise absent. Apart from its usually smaller size, the only obvious character which distinguishes *I. bütschlii* in the living state is its nucleus, which is almost invisible—in fact, often quite invisible, in organisms containing much food. In this respect it differs greatly from *E. coli*, whose nucleus—appearing like a beaded ring—is so conspicuous in the living amoeba. As a rule, *I. bütschlii* becomes rounded and begins to degenerate very soon after leaving the human intestine.

In good stained preparations this organism is easily distinguished by its nucleus from any of the other amoebae living in man. (See Pl. I, fig. 10, and Pl. II, figs. 32—34.) The resting nucleus is similar to that of many of the small free-living amoebae. It is vesicular, with a moderate-sized central karyosome, and measures from about 2·0 μ in small to about 3·5 μ in large individuals. Its diameter is usually between one fourth and one fifth of that of the whole organism—when fixed and stained; and the diameter of the karyosome varies from about one half to one third of that of the entire nucleus. The karyosome stains intensely with chromatin stains. With iron-haematoxylin it may appear homogeneous (fig. 34), or may show a paler centre (fig. 32). Occasionally a

granule can be seen in the centre—as figured by Prowazek (1912 *a*, figs. 13, 16)—but there seems no justification for calling this a centriole. I have discussed similar appearances in other amoebae elsewhere (1914), and shall not discuss the interpretation of them further here.

The nuclear membrane is fairly well developed, and stains readily. Occasionally it shows very fine granules (? chromatin) imbedded in it, but these cannot usually be seen clearly. Between the karyosome and the membrane there is the usual "clear zone," which is occupied in the present species by a layer of fairly large granules—so-called "peripheral chromatin." These granules usually lie in a single layer. They stain somewhat deeply with iron-haematoxylin, but on extraction give up the stain more readily than the karyosome. They can thus be completely decolorized, and counterstained with eosin or other plasma stains, whilst the karyosome remains deeply coloured (cf. fig. 32). In ordinary iron-haematoxylin preparations they are often overstained, so that they are confounded with the karyosome; or they may be completely decolorized, so that they disappear. Very often, in such preparations, only their outlines are visible; and this gives rise to an optical effect suggesting the presence of a network or series of septa connecting the karyosome with the nuclear membrane. This is the structure described in the nucleus of "*E.*" *bütschlii* by Prowazek. By using suitable counterstains and good lenses with proper illumination it is not difficult to convince oneself that his interpretation was incorrect, and that the clear zone is really occupied by a layer of small granules and not by a network. (Cf. figs. 10, 32, 34.) I note that Kuenen and Swellengrebel (1917) have already observed the presence of "peripheral chromatin" in the nucleus of this form. It may be noted further that these granules are generally disposed in a single layer in the active amoeba; and that they are often distinctly separated from the nuclear membrane but apparently imbedded in the karyosome, whose outline often appears slightly stellate in consequence (fig. 32).

I lay some stress on these nuclear characters, as they supply the only means of distinguishing the amoebae of this species with certainty.* All active amoebae, of whatever size, appear to possess the same nuclear structure. In very small forms, however, it is impossible to make out all the details with precision. Such organisms (fig. 33), and also many degenerate or badly fixed and stained individuals, cannot always be distinguished with certainty from similar small or degenerate specimens of *E. nana*. It may also be very difficult, or impossible, to distinguish very small specimens of *I. bütschlii* from small uninucleate individuals of *Dientamoeba fragilis*.

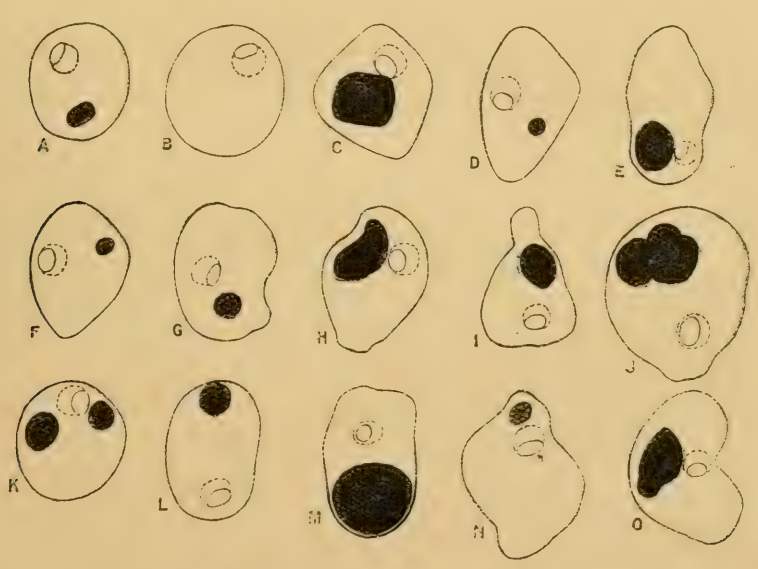
I. bütschlii appears to feed chiefly upon small micro-organisms in the intestinal contents. To judge from the inclusions in its food-vacuoles, its food habits are closely similar to those of *E. nana*.

The habitat of the organism has not been determined with certainty: but from the close parallel between the appearance of the free and encysted forms and those of *E. coli*, in the stools of persons infected with both species, I judge it to be probably—like the latter—an

* That is to say, of distinguishing them from other amoebae found in the same situation. Many of the small free-living amoebae possess, of course, a nuclear structure which is very closely similar.

inhabitant of the large bowel. *I. bütschlii* always dies very quickly outside the intestine, and has not yet been cultivated.

Nuclear "divisions" have been described by Prowazek (1912 *a*) in *I. bütschlii*, but his figures are unconvincing. I have not been able to study the division of this amoeba in detail, and the few apparently dividing nuclei which I have seen are different from those described by him. The usual method of multiplication is, no doubt, by simple bipartition—as in most other amoebae ; but stages in the process are extremely difficult to obtain. Binucleate amoebae, usually of large size, are sometimes found ; and they probably represent forms which have been arrested in division by discharge from the intestine—as in the other species. A binucleate organism has been figured by Prowazek (1912 *a*, Pl. xviii, fig. 14), but I am not certain whether this figure was really drawn from a free amoeba. The forms which I have seen usually have two resting nuclei exactly like those of the active forms, with central karyosomes : whereas his figure shows a form with nuclei like those in the cysts. He states further that multiple division occurs, but his figures are capable of a different interpretation (*vide infra*). I believe there is no evidence of schizogony in this species.



TEXT-FIG. 2.—Outline drawings of cysts of *I. bütschlii*, in iodine solution. (Magnification 1,500 diameters.)

Cysts.—The cysts of *I. bütschlii* are very remarkable structures, differing considerably from those of the other intestinal amoebae of man. They have become familiar to many workers since Wenyon first described them—as "I. cysts"—from the stools of dysenteric patients from Gallipoli in 1915. The living cysts may be nearly spherical or oval, but they are very frequently irregular in outline. They may be kidney-shaped, pear-shaped, fusiform, rhomboidal, or almost triangular or square in outline, and their forms are often such as almost to defy description. Text-fig. 2, A to O will give some idea

of the shapes commonly met with. Oval or spherical cysts usually measure about 9 μ to 12 μ in diameter : but it is extremely difficult to state the dimensions of the more irregular forms even approximately. Attempts to measure the more regular cysts have shown that their commonest size is about 9-10 μ in width by 10-12 μ in length. I have generally found that the simplest way to gauge the size of these cysts, in practice, is to measure their greatest length and their greatest width, and then take the mean as the "size" of the cyst. If this is done for a number of specimens it will usually be found that the average "size" lies between 10 μ and 11 μ. Cysts of all sizes from about 6 μ to 16 μ occur, however, but those with such extreme dimensions are uncommon.

The living cysts have a fairly thick wall. They are of a clear white colour, and usually contain two clearly visible inclusions—a number of very brightly refractile granules, and a dull area of variable size, and usually of a more or less spherical shape. The granules may be few or many, collected together or dispersed through the cyst. They resemble micrococci, and range in diameter from 0·25 μ or even less up to about 1μ. After fixation they take up nuclear stains, but give them up on extraction more readily than the chromatin in the nuclei, and then readily stain with plasma stains. They may show metachromatic staining (red) with methylene blue or haematoxylin, but their reactions are difficult to study on account of the impermeability of the living cysts to most staining reagents. Neutral red, when it can be got to enter the cyst, colours them bright red ; but as a rule the contents of fully-formed cysts remain quite colourless in watery solutions of this stain. The bright granules do not stain with Best's carmine, and as a rule stain feebly with borax carmine or paracarmine. They are insoluble in water, alcohol, chloroform, acetic acid, and most ordinary reagents. It appears to me probable, therefore, that they are not chromatin granules, and that they are chemically different from the chromatoid bodies or granules in the cysts of the *Entamoebae.* They seem, on the other hand, to consist of a substance similar to volutin, and I shall therefore speak of them as volutin granules for the present. They are shown in the cysts figured in Pl. I, fig. 11, and Pl. II, figs. 38, 40-42. They have been omitted, however, from the cysts outlined in Text-fig. 2.

The dull inclusion in the living cysts of *I. bütschlii* is very strikingly stained when they are placed in an aqueous solution of iodine. It then assumes a dark mahogany colour, and appears as a solid body with a well-defined outline. (See text-fig. 2, A to O.) This reaction at once suggests that the inclusion is a mass of glycogen ; and this is confirmed by its equally well-marked reaction when treated with Best's specific carmine stain for this substance (fig. 39, Pl. II). It is, moreover, insoluble in alcohol and chloroform, but readily soluble in water ; so that in cysts stained in watery solutions—such as iron-haematoxylin or haemalum—it is completely extracted, and its place is represented by a vacuole (cf. figs. 40-42, Pl. II). This glycogen mass is, of course, the structure which Wenyon has termed the "iodophilic body," and from which he named the cysts "Iodine cysts." It is doubtless homologous with the similar glycogen masses or vacuoles found in the cysts of *E. coli*, *E. histolytica*, *E. nana*, and other protozoa.

The glycogen masses in the cysts of *I. bütschlii* are most readily studied in cysts suspended in iodine solution. (See text-fig. 2, A to O.) They may be very small (fig. 2 F) or even absent (fig. 2 B), very large

(figs. 2 J, M), and of variable shape. Usually they are more or less rounded. At times two (fig. 2 K) or even three separate masses of glycogen may be present in a cyst. They gradually disappear from the cysts if they are kept for some days, being apparently absorbed. It thus seems probable that they represent a reserve of food material.

In addition to the volutin granules and the glycogen mass, the cyst of *I. bütschlii* contains a single nucleus. This differs in structure from that of the active amoeba, and to understand the difference it is necessary to study the changes which occur during encystation. I shall therefore revert to the active form at this stage, in order to describe this process.

Unlike *E. histolytica* and *E. coli*, *I. bütschlii* does not undergo any diminution in size prior to encystation. The precystic amoebae are, in fact, some of the largest forms of the organism met with ; so that they are not, in this respect, comparable with the "*minuta*" forms of *E. histolytica*. Amoebae which are preparing to encyst get rid of all the food contained in their vacuoles, and their cytoplasm becomes beautifully clear and transparent. At the same time the nucleus increases in size (see figs. 35, 36, Pl. II). This increase is chiefly noticeable in the zone between the karyosome and the nuclear membrane. This zone, which contains the layer of granules of "peripheral chromatin" in the active amoeba (figs. 32, 34, Pl. II) now becomes filled with much more numerous granules, often forming several distinct layers (figs. 35, 36). At this stage the nucleus has increased from about 2—2·5 μ in the active form to 3 μ or even 4 μ in diameter. The amoebae are now strikingly different from the vegetative forms. Their protoplasm is clear and white in the living organism, and in well fixed and stained specimens appears finely alveolate and remarkably uniform (figs. 35, 36). These amoebae are very sluggish, soon become non-motile, and then more or less rounded, when they secrete their cyst walls (fig. 37).

With the formation of the cyst wall further changes take place within the organism. The encysting, or partly encysted, amoeba shows at first a small and diffusely stained brown patch in its protoplasm when examined in iodine solution. This is the forerunner of the glycogen mass, and it can be seen to become larger, more deeply stainable, and with a definite contour, in individuals at later stages in development. Simultaneously the volutin granules appear in the cytoplasm. At first they are extremely small, and indistinct, but later they are seen to be larger and highly refractile. At the time of their formation they do not seem to have any definite relation to the nucleus or the glycogen mass, but make their appearance in any part of the cytoplasm. The most striking change occurs, however, in the nucleus. The karyosome, which is central in the active and precystic amoebae (figs. 32-36), gradually passes towards the periphery (fig. 37), until it lies as a large and compact mass in contact with the nuclear membrane in the fully-formed cyst (figs. 40-42). Fig. 37 shows an organism which has just formed its cyst wall. The volutin granules are already fairly numerous : the glycogen mass was small, and is represented by a small vacuole in the stained specimen. The karyosome is passing to the periphery of the nucleus, and the abundant "peripheral chromatin" has a characteristic appearance. Comparison of this figure with fig. 36—an earlier stage—and fig. 40—a mature cyst—will show at a glance the chief changes which occur during encystation.

In the karyosome of encysting amoebae a differentiation into a paler cortical part and a more deeply staining central body can often be seen in iron-haematoxylin specimens (cf. figs. 36, 37). This is not usually visible in the mature cyst. In the latter, the nucleus has the appearance of a signet-ring—especially in cysts examined in iodine (text-figs. 2A to O), in which medium the peripheral granules are invisible, though the karyosome and the nuclear membrane are usually to be made out with ease. The granules in the nucleus of the cyst often stain very intensely, so that it is necessary to stain the cysts very carefully in order to obtain a correct picture of the structure of the nucleus. Sometimes one or more of the granules will retain the stain more strongly than the remainder, so that occasional dark granules may be seen lying among the others (cf. fig. 42).

The cysts of this amoeba are very apt to undergo shrinkage during fixation, staining, and mounting; and there is thus often a space between the contents and the wall in mounted specimens. Very generally, also, there is some shrinkage of the cytoplasm surrounding the nucleus, so that the latter often appears—in stained preparations—to lie in a vacuole (cf. figs. 38-40, 42). These appearances are, of course, artifacts; but the remarkable forms of the cysts encountered in good preparations—such as figs. 41 and 42, for example—are not the result of fixation or other manipulation. They are equally visible in living cysts. Fig. 38 shows a badly fixed cyst of large size, in which there has been much cytoplasmic shrinkage. I have drawn it because it shows a common appearance of these cysts in stained preparations. It bears a striking likeness to the cyst of "*Entamoeba*" *bütschlii* depicted by Prowazek and said to measure 14·8 μ (1912 *a*, Pl. xviii, fig. 21); and consequently, it supports my identification of this form with that here described.

Cysts of *I. bütschlii* containing more than one nucleus are very uncommon. Brug (1917) states that most unusually large cysts are binucleate, but this is incorrect. I have seen several containing two nuclei, but none with more. I regard them as abnormal supernucleate forms, like the 8-nucleate cysts of *E. nana* or 16-nucleate cysts of *E. coli*. I think Prowazek's binucleate and trinucleate "amoebae" (his figures 14 and 15) are really similar cysts, of irregular form, and not stages in division or schizogony. It may be noted that James (1914) has interpreted Prowazek's fig. 15 as an enucleate amoeba of *E. coli* containing three ingested yeasts—not three nuclei. This seems to me a very far-fetched explanation.

The remarkable forms so often assumed by the cysts of *I. bütschlii* would seem to indicate that they are formed under some peculiar conditions of stress or pressure. An amoeba naturally tends to assume a spherical form when at rest, and about to encyst: and it has occurred to me that the strange shapes of the cysts of this organism may possibly be due to the fact that the amoebae are crowded together in the crypts in the large intestine at the time of their encystation, so that their cysts become distorted by mutual pressure. Similar strangely shaped specimens of other protozoa, such as coccidia, which are normally oval or spherical, may often be seen in cases of heavy infection where they are closely packed and pressed together in the tissues.

The mature uninucleate cysts of *I. bütschlii* undergo no further development outside the human body. They will remain unchanged—except for the disappearance of the glycogen, as already noted—in faeces

or water for two or three weeks; but they are unable to withstand drying, like those of the other intestinal amoebae. Their development in a new host has still to be determined. Doubtless they hatch in the small intestine, and each liberates a single uninucleate amoeba.

Descriptions and figures of the cysts of *I. bütschlii* have already been given by Wenyon (1915 *a*), Wenyon and O'Connor (1917), Kuenen and Swellengrebel (1917), Brug (1917, 1919), and Flu (1918). All have noted their striking appearance in iodine solution, but only Kuenen and Swellengrebel have stated that the "iodophilic body" is composed of glycogen. Wenyon and O'Connor note that they vary greatly in size and shape, and give their size as 7 μ to 15 μ "or more." They do not mention the volutin granules in the cysts; but Kuenen and Swellengrebel observed them, and apparently considered them to represent the "peripheral chromatin" of the nucleus extruded into the cytoplasm. Brug (1917) also noted these granules, but was unable to interpret them. He calls them "coccoid corpuscles," and says they may be parasites, "saprophytes" (*sic*), or metabolic products. He describes the "peripheral chromatin" of the nucleus as being disposed in the form of a crescent—an appearance resulting, apparently, from defective fixation. The size of the cysts is given by Kuenen and Swellengrebel (1917) as 10 μ to 12 μ—the same size as their amoebae. Flu (1918) describes the cysts as "spherical bodies with a large vacuole, which . . . assumes a more or less brown colour" in iodine solution. His figures are very poor, and he considers the "bodies"—as already noted—to be degenerate cysts of *E. histolytica*.

I believe that there are different races of *I. bütschlii* which are distinguishable by the sizes of their cysts—as in *E. histolytica* and *E. coli*. Certainly a distinct difference is sometimes observable in the size of the cysts passed by different persons. But the cysts are so difficult to measure that I cannot place much confidence in such measurements as I have made, and I am therefore unable to offer any proof of the existence of such races at present.

Occurrence.—There can be no doubt that *I. bütschlii* has a wide geographical distribution. It occurs in Egypt (Wenyon and O'Connor), the South Seas (Prowazek), the Dutch East Indies (Kuenen and Swellengrebel, Brug, Flu), and I have found the cysts in the stools of patients who have been invalided to England from all the chief areas of the present war. The infected cases examined have included persons from all the five continents; but it is, of course, impossible to discover where or when their infections were acquired. The organism occurs also in persons who have never left the British Isles.*

Wenyon (1916) found the cysts of *I. bütschlii* in the stools of 29 out of 556 cases of dysentery and other intestinal ailments invalided to England from Gallipoli in 1915. They have since been found in a similar proportion of cases of dysentery, etc., from all the theatres of war, by many other workers in England. My own records show that they must occur in at least 5 per cent. of all the cases examined, but the exact incidence I cannot determine: for different series of patients have been examined with different degrees of thoroughness, and I have examined many stools selected because they contained the cysts,

* Cf. Matthews and Malins Smith (1919).

which have often been sent to me for identification. The largest series of cases from which I can form an approximate estimate of the prevalence of this organism is that which I examined at Epsom (*vide* Dobell, Gettings, Jepps, and Stephens, 1918). Here I examined 1,300 men and found 29 infections. As the cases were examined on the average only twice each, it is certain that more were really infected. If the results can be interpreted—as seems highly probable from the figures at my disposal—in the same manner as the findings of *E. histolytica* (*vide* Dobell, 1917), then the whole series was probably infected to the extent of some 4 or 5 per cent. My figures also appear to indicate that infections with *I. bütschlii* are commoner in persons who have been in the tropics and the Near East than in those who have been in France and the British Isles only.

Wenyon and O'Connor (1917) have published statistics showing the incidence of infection in various groups of people examined by them in Egypt. The figures range from 2 per cent. up to 14·8 per cent.—the latter being the figure for natives in Hadra Prison. The percentages are derived from series examined only once per case on the average, and are therefore all too low. They were—with one exception—always lower than the percentage infections with *E. histolytica*. In my experience the frequency of *I. bütschlii* as compared with *E. histolytica* infection for the same group of cases lies as a rule between 1 : 3 and 1 : 5. It is certainly the least common of the intestinal amoebae of man (except *Dientamoeba*), but probably occurs—so far as I can judge—in at least some 3 to 5 per cent. of all human beings.

It is a curious fact that *I. bütschlii* very frequently occurs in company with *E. histolytica*—far too frequently for it to be due to chance. When not accompanied by this species, it is generally in company with *E. coli* or *E. nana*, and all the four species occur together fairly often. I have not yet found with certainty a single pure infection with *I. bütschlii*. There can be no doubt that it is an entirely independent species, however ; and I can offer no plausible explanation of its almost invariable association with other amoebae.

Pathogenicity.—Although *I. bütschlii* has usually been found in the stools of persons who have previously suffered from dysentery or other intestinal disorders, this is probably merely because the stools of healthy people are not usually investigated. I have found infections in healthy people with no history of dysentery or persistent diarrhoea. Wenyon and O'Connor (1917) have found the cysts in the faeces of healthy white troops and natives in Egypt, and Matthews and Malins Smith have found them in residents in the British Isles. There is at present no evidence that the organism is pathogenic, and it seems almost certain that it is not.

Treatment.—One of the most remarkable characters of *I. bütschlii* is its prompt disappearance when emetine is administered to its host. Wenyon and O'Connor (1917) noted that emetine hydrochloride given either hypodermically or by the mouth to infected persons causes the cysts to vanish from their stools. In none of their cases did they subsequently reappear during the period of observation. I have found that the administration of emetine bismuth iodide has the same effect, and have already recorded the apparent cure of 5 cases by this means (*vide* Dobell, Gettings, Jepps, and Stephens, 1918). I have studied other cases, both before and since, and some who were treated with emetine

hydrochloride hypodermically. In no case have I found any evidence of the persistence of this amoeba after the administration of a thorough course of emetine in any form. To judge from my own records, *I. bütschlii* may even respond more readily to emetine than *E. histolytica*, for I have never yet seen a case "relapse" after treatment. This may be a coincidence, however, as I have studied only about a dozen for a sufficient period : and, moreover, all these cases were infected with *E. histolytica*—for which the emetine was given—and were all apparently cured of infection with this parasite at the same time.

This behaviour of *I. bütschlii* towards emetine administered to its host is to me extremely puzzling. If emetine were an "amoebicidal" substance, having a specific lethal action upon amoebae generally—as was once believed—such a reaction might even have been predicted : but since it is now highly probable that emetine acts primarily upon the host, and not upon the parasite (cf. Dale and Dobell, 1917), it is difficult to understand how it eradicates infections with *I. bütschlii*. The fact that emetine will not cure an infection with *E. coli* or *E. nana* appeared to be clearly correlated with the difference in habit of these species as compared with *E. histolytica*—neither of them preying directly on its host. But in *I. bütschlii* we appear to have an organism which lives upon the intestinal contents, like *E. coli* and *E. nana*, but which responds to emetine treatment like the tissue-parasite *E. histolytica*. This may conceivably indicate that there is some peculiarity in the habits of *I. bütschlii* which has not yet been discovered, or that emetine is directly toxic to it. At present the facts seem inexplicable.

VIII.

GENUS *DIENTAMOEBA* JEPPS & DOBELL, 1918.

THERE is only one species of this genus known—*Dientamoeba fragilis*, which I have already described in detail in a joint paper (Jepps and Dobell, 1918). I shall, therefore, merely summarize here as briefly as possible what has there been said.

History and Nomenclature.

D. fragilis was probably discovered, though it was not described, by Wenyon in 1909. It was later rediscovered independently by Miss M. W. Jepps and by myself in 1917. We described seven cases of infection, which, with Wenyon's original case, make 8 known cases in all. I have found no others since, and, so far as I am aware, none have been recorded.* The organism is probably rare, but not so rare as these figures appear to indicate; for it is extremely delicate, and perishes soon after leaving the body. Consequently, infections must often be overlooked. The nomenclature has been fully discussed in my earlier paper with Miss Jepps, and need not be reconsidered here. There can be no doubt that this organism is generically distinct from all other amoebae of the human gut.

Description.

D. fragilis is a very small amoeba. When rounded it measures from 3·5 μ to 12 μ in diameter, its usual size being about 8-9 μ. It is thus similar in size to *E. nana* and the smallest precystic forms of *E. histolytica*.

The living amoebae are actively motile. They show a well-marked differentiation between their ectoplasm and endoplasm. The pseudopodia are typically composed almost entirely of ectoplasm, and are few, flattened, leaf-like, and often lobed or indented. The endoplasm is granular, and usually contains numerous food-vacuoles; but a contractile vacuole, as in other parasitic amoebae, is absent. During locomotion the animal has a somewhat snail-like appearance—the hyaline pseudopodia being in advance, the granular endoplasm concentrated in a fairly definite rounded mass posteriorly. Like other intestinal amoebae, the organism is colourless.

The most characteristic feature of *D. fragilis* is its nuclear system.

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* Since the publication of the description of this organism five cases of infection have been reported to me privately by others. On investigation none of these proved to be infections with *Dientamoeba* in reality. I record them to show the errors which can occur in diagnosis. Two of them were small free-living "*limax*" amoebae, in stale stools. Two others were leucocytes, in stools containing pus; and the remaining one was an infection with *E. nana*, in which many of the amoebae were parasitized by *Sphaerita*—the spore-morulae of which were mistaken for nuclei.

Each individual is typically binucleate (figs. 90, 91, Pl. V), the two nuclei being identical in size and structure. Their size is proportional to that of the whole organism, and their diameters range from about 0·8 μ in very small amoebae up to about 2·3 μ in very large. On the average the nuclei measure about 2 μ, in stained specimens. They are invisible or inconspicuous in the living organism; but in well fixed and stained individuals, obtained immediately after they have left the human body, they have a very characteristic structure—unlike that of the nuclei of any of the other intestinal amoebae of man. Each nucleus is spherical and vesicular. The chromatin is all situated in a fairly large central karyosome, which, in well-differentiated iron-haematoxylin preparations, can be seen to consist of a number of granules apparently embedded in a plastin matrix (see figs. 90, 91). The granules are not always uniform in size, and one of them is often larger and more conspicuous than the others (cf. the lower of the two nuclei in the amoeba depicted in fig. 91, Pl. V). No centriole is demonstrable in the karyosome. The whole nucleus is bounded externally by a very delicate and feebly staining nuclear membrane, separated by a clear zone from the karyosome. Linin threads of extreme tenuity can be seen, in well stained individuals, radiating from the karyosome to the nuclear membrane. As a rule, excessively minute granules can be resolved on or in the nuclear membrane—usually at the points where the linin threads are attached to it. Apart from these granules, of doubtful composition, there is no "peripheral chromatin" either in the clear zone outside the karyosome or on the nuclear membrane. The structure of the nuclei will be evident from inspection of figs. 90-92 (Pl. V).

The two nuclei of a *Dientamoeba* may occupy any position, relatively to one another, in the body of the organism. They are sometimes in contact (fig. 91), or may be separated by an interval of variable extent (fig. 90).

Most individuals are binucleate, but in every infection a certain number of uninucleate specimens can always be found (fig. 92). Careful examination of over 1,000 individuals from three different cases showed that about 80 per cent. of them were binucleate and 20 per cent. uninucleate. Uninucleate individuals may be found of all sizes, from the smallest to very large; but they are, on the whole, somewhat smaller than the binucleate forms. These, however, are sometimes of extremely small size (3·5 μ).

Division stages are extremely rare in the stools, and all that have so far been found represent organisms with a single dividing nucleus. From this fact—and others just noted—I have been led to suppose that *D. fragilis* differs from the other known binucleate species of amoebae (such as "*Amoeba*" *binucleata* Gruber, and "*Amoeba*" *diploidea* Hartmann et Nägler) in its mode of reproduction. It seems probable that the organism is, when full grown, binucleate; and that when division occurs, it consists in a simple fission of the cytoplasm, resulting in the formation of two uninucleate daughter individuals. The nucleus in each of these divides into two during the growth period, thus giving rise to the adult binucleate form once more.

D. fragilis undergoes degeneration in a very characteristic manner. Soon after leaving the body the amoebae usually become rounded and motionless, and filled with vacuoles. These then coalesce into a single large central vacuole, surrounded by a thin layer of protoplasm. The

organism then appears under the microscope as a delicate ring of protoplasm, containing the two nuclei and any food inclusions which may have been present, with a large space in the middle. At this stage it has a striking resemblance to a *Blastocystis*. Degenerating organisms may remain in this condition for a long time before they finally disintegrate.

Although a prolonged and very careful search has been made for the cysts of this organism, none have ever been found. In this respect *D. fragilis* resembles *E. gingivalis*. How the former is transmitted from host to host is still a matter for speculation. Only when the stools of an infected individual are liquid or soft can the amoeba be found in them. When they are hard and formed, no trace of the amoeba, and no resistant stages, can be detected. The free amoeba usually perishes so rapidly outside the body that it is highly improbable that it can be transmitted in this form.

The habitat of *D. fragilis* is probably the colon, but up to the present it has been found only in the stools. It is not known to occur in any animal but man, and has not yet been cultivated.

To judge from the food contained in its vacuoles, *D. fragilis* lives exclusively on bacteria and other small vegetable micro-organisms in the gut contents. It is therefore, in all probability, a harmless commensal like *E. coli* and *E. nana*. No method of treatment is known to have any effect upon it.

The geographical distribution of this remarkable and interesting organism is at present uncertain, but it seems probable that it is wide. Of the seven cases of infection which I have studied, one was in a healthy resident in Great Britain, who had never been abroad; two were in British soldiers invalided home to England from Salonika, suffering from dysentery; one was in a British soldier invalided from Salonika to England with malaria; and the remaining three cases were New Zealand soldiers who had served in France and England only. Although most of these cases had suffered from dysentery, there is no evidence that it was in any way due to infection with *D. fragilis*: and none of them were suffering from dysentery at the time when their infections were discovered.

IX.

THE AMOEBAE FOUND IN HUMAN URINE, IN DOGS, AND IN MONKEYS.

I HAVE already mentioned that there are several problems connected with the amoebae which have been described from human urine, and also with those which have been found in certain animals other than man—especially dogs and monkeys. It is not possible to deal thoroughly with several problems in the nomenclature of the amoebae of man without taking these organisms into account; and I shall therefore say something about all of them in this section, and endeavour to clear up some of the difficulties which they offer at present.

I shall deal with the amoebae from urine, from dogs, and from monkeys, separately and in this order.

THE AMOEBAE FOUND IN HUMAN URINE.

There are now over a dozen records of amoebae discovered in the urine or urinogenital organs of human beings, and a condition of "urinary amoebiasis" is recognized by some clinicians. Since the amoebae concerned are supposed by some workers to be *E. histolytica*, and since they have received various names, it has already been necessary to notice them when dealing with the nomenclature of this species.

Amoebae appear to have been first recorded in the urine by Baelz (1883), who found them in a young Japanese woman with "tuberculosis of the urinogenital apparatus and lungs." She entered hospital the day before her death. Her urine, removed by catheter, was bloody and contained much pus and necrotic tissue; and, in addition, Baelz found enormous numbers of actively motile amoebae measuring 50 μ in diameter, and, except for their rather larger size, "in every way" like Lösch's "Amoeba coli." According to Baelz, "they consist of a granular body-substance with a large vesicular nucleus": and he says they were present in the bladder and vagina, and he believed that they had wandered from the latter into the former. He does not record that they contained ingested red corpuscles, and apparently he did not examine the stools. No *post mortem* examination was made. He remarks that "if the parasite, as is probable, represents a new species, then it may be given the name Amoeba urogenitalis." Blanchard (1885), in recording this case, names the organism "*Amoeba vaginalis* Baelz, 1883"—which is incorrect, and presumably a *lapsus calami*.

Jürgens (1892) has described mucous cysts "filled with amoebae" which he found in the bladder of an old woman—a case of chronic cystitis and endometritis. The "amoebae" are not properly described: but it is stated that they put out pseudopodia when warmed, and that some of them had no nuclei. Their resemblance to cells was noted, and

they are said to have been present in the vagina as well as the bladder. I am at a loss to know why these structures were supposed to be amoebae. It seems highly probable that they were cells, though of what sort precisely one cannot determine from the inadequate description.

Kartulis (1893, footnote p. 2) says he found "amoebae" in the urine of a Cretan, but he gives no proper description of them—merely noting that they measured 12-20 μ, had "finely granular" protoplasm, and showed a nucleus in stained preparations. His reasons for regarding these bodies as amoebae are not given.

Posner (1893), in Berlin, describes and figures "amoebae" found in the urine of a man with haematuria. They changed shape slowly, were not motile, and usually measured 25-30 μ in diameter. Although the author speaks with confidence, and says that they "immediately struck everyone who saw them as amoebae," his figures and descriptions are not likely to impress anybody familiar with these protozoa. There can be no doubt, I think, that the objects he saw were really cells. At all events, there is no evidence that they were amoebae. Wijnhoff (1895) studied four similar cases of "urinary amoebiasis" at Utrecht: but although he describes "amoebae," "cysts," and "multiplying" forms, it is clear from his account that the structures which he encountered were not amoebae but cells of various sorts occurring in the urine.

Craig (1911, p. 233) mentions that he has studied a case of urinary amoebiasis. The bladder is said to have been infected with *E. histolytica*, and a minute fistula between it and an intestinal amoebic ulcer is said to have been detected *post mortem*. No details are recorded.

Fischer (1914) found amoebae in the urine of a Chinaman at Shanghai. After one visit to the hospital—when the amoebae were found—the patient unfortunately left. No proper history was obtained, and the case was not followed up. A diagnosis of "? Cystitis" was made. The stools were not examined, and it is uncertain whether the patient had ever had dysentery. The amoebae are stated to have been actively motile and much vacuolated, and measured about 20-25 μ in diameter. They were not properly described, but are said to have been indistinguishable from "*Entamoeba tetragena*," as seen in local cases of amoebic dysentery. It is not stated whether they contained red corpuscles or not, and their mode of entry into the urine was, of course, undetermined.

Lynn (1914) has recorded the case of "an intelligent coloured man" who washed out his rectum and then his bladder with the same syringe—without sterilizing it—with the result that blood and pus appeared in his urine, and "urinalysis" revealed "motile amoebae (*Entamoeba tetragena*)," according to a laboratory report. No amoebae were found in the stools. This case is said to have "responded" to emetine treatment—though the evidence for this is, to say the least, meagre. In the absence of any further evidence concerning the "amoebae," it is impossible to accept them as *E. histolytica*.

Walton (1915) has published an important paper dealing with a case of urinary amoebiasis. His patient was an Indian native with chronic Bright's disease, whose urine contained very numerous highly active entamoebae. Nearly all of these contained red blood corpuscles. Rounded specimens measured usually 25-35 μ in diameter in stained preparations. No cysts were found. The amoebae are carefully described, and the author concludes that they were "*E. tetragena*." Amoebae, apparently of the same species, were found in the stools.

The case was treated with emetine hydrochloride hypodermically (1 grain daily), and on the fifth day the amoebae disappeared completely and were not found again, though the case was carefully controlled for over a month. An attempt to infect a kitten *per anum* with amoebae from the urine gave negative results, as also did a similar experiment with the amoebae from the stools.

Macfie (1916) describes one case in a European, and records four others in natives, of urinary amoebiasis seen on the Gold Coast. The parasites were found in the urine, but never in the stools. They were generally "quiescent," but sometimes showed sluggish movements; and they were usually degenerate, owing, it is suggested, to the harmful action of urine on them. Sometimes they contained red blood corpuscles. They measured 7-33 μ in diameter, but 50 individuals measured on one occasion gave an average of only 10 μ. The nucleus possessed "a well-defined nuclear membrane, refractile, and clearly differentiated from the surrounding endoplasm. On the inner side of this membrane there was usually a large amount of chromatin arranged in irregular nodular masses, and throughout the nuclear substance similar chromatic matter was distributed. The karyosome was large and showed a centriole." It is difficult to see much resemblance to *Entamoeba histolytica* in this account, but the author states it as his belief "that these amoebae cannot be differentiated from *Entamoeba histolytica* (*tetragena*)." He also states that he found cysts in the urine, "but none was seen containing more than four nuclei." These "cysts" are not figured—nor, indeed, are the amoebae. (It may be recalled that the "cysts" of amoebae which Macfie (1915) described previously from a monkey are clearly shown by his figures to have been *Blastocystis*.) Dr. Macfie very kindly sent me some preserved sediment from the urine of one of his cases. I have examined it very carefully, but have been unable to find anything but pus and various tissue-cells in it. No recognizable amoebae, and nothing even resembling cysts of *E. histolytica*, have rewarded my search. I am therefore still unconvinced that the structures which he observed were really amoebae.

Ward, Coles, and Friel (1916) discovered "amoebae" in the urine of a case of jaundice, and proposed to call them "*Amoeba urinae granulata*." From their account, and the later revelations of Friel (1917), there can be no doubt that the bodies in question were not amoebae at all. It will suffice to note that "the bodies were totally unlike *Endamoeba coli* or *Endamoeba histolytica*" (Ward, Coles, and Friel, 1916); and that "it is probably not an amoeba in the proper sense of the term," but "resembles a vegetable cell, recalling the 'protococcus' met with in stagnant water" (Friel, 1917).

Chalmers and O'Farrell (1917) describe a case of "urinary amoebiasis" in a Greek woman in the Sudan, and mention several others in which they found amoebae "always more or less encysted, and more or less degenerate." In the case described they were non-motile, "roundish," and measured about 18 μ in diameter, with a nucleus of 4 μ. They were believed to be a "pre-cystic stage" of *E. histolytica*. On one occasion "numerous degenerating cysts appeared in the urine." The authors find "no reason to doubt" that their identification was correct: but their description and figure (a photomicrograph) convince me that they were mistaken. I cannot recognize *E. histolytica* in either; and their belief in the patient's "response" to emetine treatment seems by

no means justified by the facts given. It is important to note also that the faeces were twice examined carefully (after a purgative), and no amoebae were found. Equally unconvincing to me is the account of "amoebae" found in the urine of a woman at Sierra Leone by Wright (1917). They were usually "quiescent," and measured usually 20-25 μ in diameter. Cysts "were numerous," and many had "2 and 3 nuclei." The "cysts" had a diameter of about 20 to 25 μ. Although the author states that he "is persuaded to regard the amoebae as belonging to the 'histolytica' type," I am unable to find any grounds for such persuasion: nor is the statement that the patient "responded readily to specific treatment with emetine hydrochloride" likely to command assent from anybody who reads the recorded details in a critical spirit.

Finally, Aravantinos and Michailides (1918) record the finding of amoebae in the urine of a Greek boy with cystitis. They are said to have been active, and uninucleate; but they are not described or figured, and were not investigated cytologically. The authors consider that the amoeba was not *E. histolytica*. The case is supposed to have been cured by lavage of the bladder with an infusion of ipecacuanha. During treatment "cysts" appeared in the urine, but they are not described. This is another very unsatisfactory case.

It appears to me probable that most of the cases of "urinary amoebiasis" hitherto described should be rejected, since there is not sufficient evidence that the "amoebae" which were found really were amoebae. There is, in fact, little to indicate that the bodies were even protozoa in most cases—still less that they were amoebae or that they belonged to any particular species. For my own part, I have little doubt that Jürgens (1892), Kartulis (1893), Posner (1893), Wijnhoff (1895), Macfie (1916), Chalmers and O'Farrell (1917), and Wright (1917) really found cells of various sorts and mistook them for amoebae. The "amoebae" of Ward, Coles and Friel (1916) and Friel (1917) are self-condemned; whilst the "amoebae" of Craig (1911) and Lynn (1914) cannot be discussed in the absence of all descriptions of them. The "amoebae" of Aravantinos and Michailides (1918) were possibly *Trichomonas vaginalis*. The degenerating amoeboid forms of this flagellate can, as I know from experience, be mistaken for "amoebae," and they occur in the bladder and urethra of males as well as of females. It is possible that some of the other "amoebae" described were also this organism in reality.

By far the most satisfactory case of urinary amoebiasis yet recorded seems to me to be that of Walton (1915). I see no reason to doubt that he really found amoebae, and that these were—as he believed—*E. histolytica*. There is no reason why this parasite should not occasionally occur in the urine, though infection of the urinary system must always be secondary to a primary infection of the large intestine. If *E. histolytica* is found in the urine, therefore, a concomitant infection of the bowel should also be present. This was so in Walton's case; but the absence of amoebae or cysts from the stools of some of the other cases speaks against the supposition that the "amoebae" in the urine really were *E. histolytica*. It is against all analogy to suppose* that the organisms

* This I take to be the explanation which Macfie (1916) offers to account for the absence of an intestinal infection in his case. He says he is "inclined to believe . . . . that this patient may at some time have unconsciously harboured amoebae in his large intestine," and that they somehow "found their way . . . . to the neighbourhood of the seminal vesicles," and so emerged by way of the urethra.

would migrate *en masse* from the gut to the secondary site of infection. Moreover, if the urinary system were secondarily infected, one would not expect to find cysts of the parasite in this situation. It is a general rule that *E. histolytica* does not encyst in the secondary sites of infection, but only in the primary site in the bowel. The occurrence of cysts in the urine, therefore, as recorded by Macfie (1916), and Chalmers and O'Farrell (1917), would—if a correct observation—be surprising. On *a priori* grounds it seems to me to weaken rather than strengthen their case. The "cysts" of Wright (1917) were clearly, from their size and structure, not cysts of *E. histolytica*.

Walton's case * I regard as a true case of secondary infection of some part of the urinary system with *E. histolytica*. The negative experiments which he made with kittens do not invalidate this conclusion, as similar negative results can often be obtained with undoubted *E. histolytica* from stools. The case of Fischer (1914) was, I believe, in spite of its obviously deficient investigation, a similar case of *E. histolytica* infection, and I see no reason why Baelz's (1883) original case should not be included in the same category. If it be included, then "Amoeba urogenitalis" Baelz, 1883, becomes a synonym of *E. histolytica*—which is, I think, the most justifiable interpretation at present. *Amoeba vaginalis* Blanchard, 1885, being another name for the same organism, is then likewise a synonym of *E. histolytica*.

I regard Baelz's name "Amoeba urogenitalis" as a provisional descriptive term and not as a Linnaean name subject to the law of priority. He did not determine whether his organism was a new species or not, and his name was proposed in case it should turn out to be new. Chalmers and O'Farrell (1917) refer to "the unpleasant point" that "if the rules of zoological nomenclature are pressed, we ought to call the amoeba of dysentery by Baelz's name." Unfortunately they do not state how they arrive at this conclusion, which seems to me wholly without foundation. If Baelz's name is regarded as valid, then Lösch's name ("Amoeba coli") has at least an equal right to recognition. Now the latter was undoubtedly given to the dysentery amoeba. Baelz's amoeba either was or was not the same organism. If it was, and "the rules are pressed," then *Amoeba urogenitalis* Baelz, 1883, is a synonym of *Amoeba coli* Lösch, 1875, and therefore cannot be used. If Baelz's amoeba was not the same species as Lösch's, then it was not the dysentery amoeba, and *Amoeba urogenitalis* is the name of a different organism. It therefore appears to be impossible to call the dysentery amoeba by Baelz's name—whatever interpretation is adopted. I hold that both these names should be cancelled, on the grounds already stated.

The Amoebae found in Dogs.

Amoebic dysentery is said to occur spontaneously in dogs. The parasite which causes it apparently resembles *E. histolytica* closely, and has been named. It is therefore necessary to consider this organism.

Kartulis (1891) says that he has seen spontaneous amoebic dysentery in a dog in Egypt; and that the amoebae were indistinguishable from those in human amoebic dysentery, and produced a similar ulceration

* I may say that I attach particular importance to this case because of my personal knowledge of the author and of his skill and competence as a protozoologist.

of the gut. Later (Kartulis, 1913) he states that he has observed two dogs with amoebic dysentery, and that one of these also developed a liver abscess. Darling (1915),* in Panama, studied a fatal case of amoebic dysentery in "a large hound used for hunting deer." The dog's colon was ulcerated, and many amoebae were present in the ulcers and in the bloody mucous stools. They resembled "*E. tetragena*"—to judge from his description, they were indistinguishable from this species (*E. histolytica*). Darling considers the possibility of his dog having acquired its infection from man, but says (incorrectly) that "dogs are not susceptible to infection by *E. tetragena*," and accordingly proposes provisionally to name the *Entamoeba* of the dog *E. venaticum*.† Ware (1916) has since described an outbreak of amoebic dysentery among foxhounds in India. Altogether nine dogs were affected, of which one died and eight were apparently cured by the hypodermic administration of emetine. The amoebae were active organisms, some of them containing ingested red blood corpuscles. "Mr. Shunker pronounced them to be extremely like entamoeba histolytica" (*sic*). They were therefore believed to belong to this species.

Now it is known that dogs can be experimentally infected with *Entamoeba histolytica* from man. Lösch (1875) injected 4 dogs "*per os et anum*" with amoebae from his celebrated original case of dysentery. One of them contracted amoebic dysentery, with typical ulceration of the bowel. Hlava (1887) also is said to have obtained positive results with 2 out of 17 dogs similarly treated, though Kartulis (1891) failed to confirm these experiments. Kruse and Pasquale (1894) record that they infected a dog with dysentery amoebae from man. Harris (1901) succeeded in infecting 3 puppies—though 4 older dogs were negative—by means of rectal injections of bloody mucous stools containing active amoebae from a human case of amoebic dysentery. All the infected animals had dysentery, with typical amoebic lesions of the bowel *post mortem*, and two of them developed amoebic abscesses of the liver in addition. Dale and I (1917) have also infected 2 puppies by rectal injection of *E. histolytica* belonging to a strain propagated through a long line of kittens.

It is thus clear that the dog, like the cat, is susceptible to infection with *E. histolytica*. In both animals infection results in acute dysentery, and may be followed by liver abscess—as in man. The dog—again like the cat—apparently does not become a carrier of the parasite: at all events, nobody has yet discovered the cysts of *E. histolytica* in the stools of infected animals, and Darling (1915) expressly notes their absence in his case. The two infected puppies which I have seen both recovered spontaneously, and neither subsequently passed cysts. They were specially examined to determine this point. One of them was finally sacrificed, and showed no signs of ulceration of its intestine.‡ It seems to me doubtful whether emetine has any curative action on amoebic dysentery in the dog—in spite of the findings of Ware (1916): for it is possible that his dogs might have recovered without treatment, and it has been found (Dale and Dobell, 1917) that emetine will not cure amoebic dysentery in the cat—which behaves in other ways similarly to

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* First recorded, but not fully described, in Darling (1912).

† This is presumably a mistake—*venatica* (or possibly *venaticorum*) being intended?

‡ *Vide* Dale and Dobell (1917).

the dog when infected with *E. histolytica*. If Ware's conclusion is correct, however, it emphasizes the resemblance between the *Entamoeba* found in the dog and *E. histolytica*.

It seems to me highly probable that the spontaneous amoebic dysentery and liver abscess observed in dogs by Kartulis, Darling, and Ware were all due to infection with *E. histolytica*. The cases all occurred in countries where human faeces containing cysts of this parasite cannot be uncommon : and the habits of the average native and the average dog are quite compatible with the hypothesis that dogs, if susceptible, may occasionally acquire an accidental infection with *E. histolytica* as a consequence of ingesting cysts deposited by human carriers. It therefore seems justifiable to conclude—in the absence of evidence to the contrary—that *Entamoeba "venaticum"* Darling is a synonym of *E. histolytica*.

The Amoebae Found in Monkeys.

Many monkeys harbour species of *Entamoeba* which are not with certainty distinguishable from those of man. As some of them have received names, it is necessary to consider them.

Kruse and Pasquale (1894) unsuccessfully attempted to infect a monkey with amoebae from a case of dysentery. Musgrave and Clegg (1904) believed that they were able to infect monkeys (*Macacus cynomolgus* and *M. philippinensis*) with amoebae from their cultures, thereby producing dysentery in them. Walker and Sellards (1913), however, were unable to infect the same species with *E. histolytica*. Musgrave and Clegg state that they "have occasionally observed cases of naturally contracted amebiasis in monkeys" in the Philippines : which may account for their "positive" results (obtained with harmless free-living amoebae), and the ulceration of the colon described and figured in their experimental animals.

Wenyon (1908) records that he found "cysts of an amoeba indistinguishable from those of *Entamoeba coli*" in "the intestine of a monkey" at Khartoum. Brumpt (1909) found a similar organism, with 8-nucleate cysts, in *Macacus sinicus*. Attempts to infect 4 cats were negative. Noc (1909) tells us further that the "macaques" of Saigon commonly pass amoebic cysts 10-12 μ in diameter in their faeces, and that they suffer from spontaneous dysentery.

Castellani (1908) described a spontaneous case of amoebic liver abscess in a Ceylon monkey (*Macacus pileatus*), and named the parasite *Entamoeba nuttalli*. He found no lesions in the bowel, and from his account and figures (from dried films) the organisms cannot be identified, as all cytological details are lacking. According to Kartulis (1913) Strong observed a case of amoebic appendicitis and liver abscess in an orang-utan at Manila. Chatton (1912 *a*) found small amoebae—many containing chromatoid bodies—in the faeces of a dead *Macacus sinicus*. They closely resembled the precystic forms of *E. histolytica*, but he found no mature cysts, and no lesions in the large intestine, and therefore did not name the organism—beyond referring it to his genus *Löschia* (= *Entamoeba*). Franchini (1912) claims to have infected "a monkey" with "Amoeba tetragena" from a human case of dysentery, but his figures and description are far from convincing. * Ujihara (1914) also claims to have infected a monkey with this species, but his experiment is very questionable.

* Some of the "amoebae" figured appear to be *Lamblia*.

Mathis (1913 *a*) has shown that the Tonkin monkeys (*Macacus rhesus* and *M. tcheliensis*) are commonly infected with two species of *Entamoeba* which appear to be indistinguishable from *E. coli* and *E. histolytica*. The one, with 8-nucleate cysts, he named *Löschia legeri;* the other, with 4-nucleate cysts containing chromatoid bodies, *L. duboscqi*. Mathis thus regarded these species as distinct from the closely similar forms in man, and Mathis and Mercier (1917 *c*) have since attempted to show that morphological differences exist between *E. coli* and *E. legeri*. They fail to prove their case, however, and advance no character of the latter which may not also be discoverable in the former.

Prowazek (1912 *a*) had previously found an *Entamoeba* in an orang-utan (*Simia satyrus*), and named it *E. pitheci*. This "species" appears to be a mixture of Mathis's two species. Later, Behrend (1914) found amoebic cysts in *M. rhesus*—said to resemble those of *E. coli*, but measuring 8-25 μ in diameter, and apparently also belonging to both the species of Mathis.

Swellengrebel (1914) has also described an *Entamoeba*, which he named *E. chattoni*, from *M. rhesus*. It appears to resemble a strain of *E. histolytica* which forms small cysts—the diameter of the cysts of *E. chattoni* being given as 8-9 μ. It is said further, however, that no cysts with more than two nuclei were ever found. Swellengrebel identifies his amoeba with that of Chatton (1912 *a*), but not with those of other authors. Macfie (1915) has recorded an amoeba from a monkey (*Cercopithecus petaurista*) on the Gold Coast. It was believed to have caused a fatal dysentery to its host, though the evidence appears inconclusive. It is said to measure 12-30 μ in diameter (stained), and to contain both bacteria and red blood corpuscles. No cytological characters of any systematic value are recorded. The organism is said to form cysts, with a diameter of 12 μ to 33 μ: but the figures of them show unmistakable specimens of *Blastocystis*. The amoeba was named *Entamoeba cercopitheci*.

Finally, Eichhorn and Gallagher (1916) in America record an outbreak of spontaneous amoebic dysentery among captive spider monkeys (*Ateles ater*). Eight showed typical amoebic ulceration of the intestine, and two developed amoebic liver abscesses. The amoebae are quite unrecognizable from the figures. The authors state that "no special attempt was made to determine the species of the amebas concerned"; and they also state that attempts to infect cats by feeding them on dysenteric stools containing the amoebae were unsuccessful. They conclude that "these negative transmission experiments suggest that the parasite found is of a different species from that in man." It is obvious, however, that such an experiment is meaningless, and would have been equally negative if they had made it with active forms of *E. histolytica*.

From the foregoing * it seems fairly clear that several different species of monkey are infected with at least two different *Entamoebae*, which are not yet distinguishable with certainty from *E. coli* and *E. histolytica*. Monkeys appear, moreover, to be subject to amoebic dysentery and

* I have not included some observations recorded by Greig and Wells (1911), who state that they were able to cultivate amoebae from the faeces of 53 monkeys (*Macacus* sp. ?); because in their tables I can find no evidence that they found parasitic amoebae in any of their animals. Craig's (1912 *a*) statement that these authors found amoebae indistinguishable from those of man in all the 53 monkeys they examined therefore appears to me to be incorrect.

amoebic abscess of the liver. It seems probable, therefore, that they harbour a non-pathogenic *Entamoeba* like *E. coli*, with an 8-nucleate cyst, and a pathogenic parasite like *E. histolytica*, with a 4-nucleate cyst. These two species, if really distinct and valid, should probably be named as follows :—

(1) *Entamoeba pitheci* Prowazek, 1912, emend.

Syn. :

Entamoeba pitheci Prowazek, 1912 (*pro parte*).
Löschia legeri Mathis, 1913.
? Entamoeba cercopitheci Macfie, 1915 (*pro parte*).
Entamoeba legeri Mathis & Mercier, 1917.

Non-pathogenic. As yet indistinguishable from *E. coli*. Observed also by Wenyon (1908), Brumpt (1909), and Behrend (1914), but not named by them.

(2) *Entamoeba nuttalli* Castellani, 1908.

Syn. :

Entamoeba pitheci Prowazek, 1912 (*pro parte*).
Löschia sp. Chatton, 1912.
Löschia duboscqi Mathis, 1913.
Entamoeba chattoni Swellengrebel, 1914.
Entamoeba cercopitheci Macfie, 1915 (*pro parte*).

Probably a facultatively pathogenic tissue-parasite—causing dysentery and liver abscess. At present indistinguishable from *E. histolytica*. Observed probably by Musgrave and Clegg (1904), Strong (*vide* Kartulis, 1913), Noc (1909), ? Franchini (1912), Behrend (1914), and Eichhorn and Gallagher (1916), but not named by any of these authors. Swellengrebel's form is, perhaps, a distinct species, but this is still unproved.

I have observed the cysts of both these amoebae in the faeces of *Macacus rhesus** which I examined in London in the course of my work with Dr. H. H. Dale (*vide* Dale and Dobell, 1917). At present I am unable to distinguish them from those of *E. coli* and *E. histolytica* respectively, and I think it by no means impossible that the amoebae are really identical with these species. If this is so, then *E. pitheci* Prowazek (with its synonyms) becomes a synonym of *E. coli* (Grassi) Casagrandi et Barbagallo, and *E. nuttalli* Castellani (with its synonyms) a synonym of *E. histolytica* Schaudinn. At present there are not sufficient data to determine this point, and the question can only be decided by further observation and experiment. There is, at all events, as yet no proof that monkeys harbour *Entamoebae* in any way different from those of man.

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* Attempts which were made to infect two of these monkeys with *E. histolytica* were negative.

X.

NOTES ON CERTAIN OTHER AMOEBOID ORGANISMS DESCRIBED FROM MAN.

I HAVE already had occasion, in the preceding pages, to notice a number of questionable amoebae which have been described from human beings. Some at least of these are probably not really amoebae, but other organisms or even cells belonging to the human body. Many of the "amoebae" found in urine, for example, undoubtedly belong to this last category. There are, however, several other "amoebae" which have not yet been discussed, but which must be mentioned in any work which aims at dealing with all the amoebae of man.

It has already been noted that the "amoebae" described originally by Lambl (1860) were, in all probability, not amoebae but *Trichomonas*. It has also been noted that the original "giant amoebae" of Kartulis (1885), from cases of dysentery, were also probably not amoebae, though what they really were I have been unable to determine. In addition, we have seen that the amoebae described by Noc (1909), Gauducheau's (1908) "*Entamoeba phagocytoides*," and several other amoebae supposed to be more or less parasitic in man, were all either free-living organisms or mixtures of these with one or other of the amoebae which really live in the human intestine. It is not necessary to say more about these "species" here, and I shall therefore now confine my attention to certain other "amoebae" which have not yet been discussed.

The Amoebae cultivated from Human Stools and Liver-abscess Pus.—It is now generally recognized that none of the amoebae of man can be cultivated in the media ordinarily used for the cultivation of free-living forms: and it will now be generally admitted that all the species obtained in cultures made from stools or from liver-abscess pus belong to the latter category. The following "species," which have been named, have been cultivated from human faeces:

Amoeba lobosa guttula, *A. lobosa oblonga*, *A. spinosa*, *A. diaphana*, *A. vermicularis*, *A. reticularis*—all described and named by Celli and Fiocca (1894 *a*).

Amoeba gruberi Schardinger (1899).

Entamoeba tropicalis Lesage (1908).

Amoeba hominis Walker (1908).

Amoeba lobospinosa Craig (1912).

There are others, but these are the species most often cited, or confused with the amoebae living in man. In my opinion not one of these species, with the exception of Schardinger's,* is identifiable from the

* This is the organism renamed "*Amoeba punctata*" by Dangeard (1910)—an easily recognizable and common form which has been frequently studied and almost as frequently named.

description given. Consequently, I think all the names will have to be abolished. There are also many other imperfectly described amoebae recorded from stools, but fortunately most of them have not been named.

Several different workers have cultivated amoebae from the pus of liver abscesses. In every case these were free-living species, and not *E. histolytica*—so far as can be determined from the descriptions. Gauducheau (1912) obtained his "*Entamoeba phagocytoides*" from this source, and Musgrave and Clegg and others had previously obtained other amoebae in a similar manner. Liston and Martin (1911) and Martin (1911) described two species of "amoebae from liver abscesses" —a "large" and a "small." Their cultures were obtained originally from Wells, in India, whose paper on "Aerial contamination as a fallacy in the study of amoebic infections by cultural methods" (Wells, 1911) probably contains the true explanation of the origin of their organisms. Liston and Martin's "small amoeba" I am unable to identify, from the incomplete description. Their "large amoeba" is—as I have elsewhere (1914) pointed out—a species closely related to the forms placed in the genus *Hartmannella* by Alexeieff (1912 *a*), of which the type is *H. hyalina* Dangeard (= *Amoeba hyalina* Dangeard, 1910).

"*Leydenia gemmipara* Schaudinn, 1896."—In ascitic fluid from two patients with abdominal malignant growths, Leyden and Schaudinn (1896) discovered some peculiar bodies which they interpreted as amoebae. To these Schaudinn gave the new name *Leydenia gemmipara;* but he stated subsequently (Schaudinn, 1903) that they were really the naked amoeboid forms of the shelled rhizopod *Chlamydophrys stercorea* Cienkowski (1876). This organism, according to Schaudinn, lives coprozoically in human faeces, but the amoeboid forms of it may be found occasionally in the intestine. "*Leydenia*" has been regarded as a very questionable rhizopod by many workers already, and I fully share their doubts.

I have never encountered *Chlamydophrys* in human faeces; and the amoeboid forms, if they ever do occur in the intestine of man, must be excessively uncommon. It should be remembered that Schaudinn recognized only two species of amoebae in man—*E. coli* and *E. histolytica:* and I suspect that his "*Chlamydophrys*" amoebae were really *E. nana*, which occurs so commonly but which he did not know. I have already pointed out that this is probably the correct interpretation of the "*Chlamydophrys*" found by Elmassian (1909), and so far as I am aware no other worker has "confirmed" Schaudinn's observation. I do not doubt that *Chlamydophrys* may occasionally occur in stale human faeces, though I have never encountered it. It certainly occurs in the dung of various animals, and in sewage beds.* But that there is any connexion between this organism and "*Leydenia*," or any of the amoebae of the human bowel, I regard as highly improbable. We do not know how Schaudinn convinced himself of the identity of these different forms, as he adduced no evidence for his statements; and it is not to be forgotten that most of his other statements about the amoebae of man were incorrect.

I have little doubt that "*Leydenia*" itself was not an amoeboid

* I have studied the organism in the faeces of frogs and toads (Dobell, 1909): and I may record that my friend the late Mr. C. H. Martin showed me, some years ago, a fine culture of *Chlamydophrys* which he obtained in material from a sewage farm.

organism at all. The "amoebae" were really cells from the body-cavity. Their movements, which consisted chiefly in change of shape and the emission of "pseudopodia," and their "contractile vacuoles"—which contracted only once in a quarter of an hour—appear to me to supply insufficient data for assuming that they must have been amoebae; whilst the "plasmogamy" and the "reproductive" phenomena (budding, division, etc.) observed and figured are far too much like aggregations of degenerate body-cells to be accepted as evidence of rhizopod affinities. Without making a careful study of the cellular elements in ascitic fluid I am not prepared to identify Schaudinn's "organisms" more precisely. But I find it impossible to regard "*Leydenia*" as an amoeba without very much stronger evidence, and its identification with *Chlamydophrys* I regard as a mere speculation.

"*Amoeba miurai* Ijima, 1898."—Shortly after the discovery of "*Leydenia*," Miura discovered a similar "organism" in a Japanese woman. It was described by Ijima (1898), who named it *Amoeba miurai*. According to him, it was present in the serous exudate from the pleural and peritoneal cavities of the patient, who was diagnosed by Miura as a case of "*pleuritis* and *peritonitis endotheliomatosa*." Two days before her death the same "organisms" were also found in the bloody mucous stools. Ijima noted the resemblance of "*A. miurai*" to "*Leydenia*," and also that it was "discovered under almost identical circumstances"; but he regarded it as belonging to a different species. No movements, save change of shape, were observed, and no contractile vacuoles; but nuclei varying in number from one to three were present. Inspection of the figures leaves no doubt in my mind that "*Amoeba miurai*" is closely similar to "*Leydenia*," and is to be interpreted in the same way: that is to say, it is not an amoeba, or protozoon of any kind, but a misinterpretation of cells from the pleural and peritoneal cavities. This "organism," therefore, should be deleted from the list of amoebae living in man.

"*Amoeba pulmonalis* Artault, 1898."—I have already mentioned—when discussing *E. gingivalis*—that Artault (1898) found an "amoeba" in a lung cavity. He proposed to call it *Amoeba pulmonalis*. It was present in small numbers in the sputum, among leucocytes, etc., and is said to have undergone changes in shape, though apparently it was not motile. Beyond the statement that the "amoeba" showed "a nucleus and a vacuole" very clearly, no account of the morphology was given. It was not figured; and the author states that it was "perhaps the same as the *Amoeba vulgaris*"—an organism with which I am unacquainted. There is nothing in Artault's description to indicate that he was dealing with an amoeba; and even if he was, there is no character given which can enable its species to be determined. It seems highly probable, indeed, that his "amoebae" were really cells.

Brumpt (1910) states that he has also observed "*A. pulmonalis*." He gives figures of the "organism" which are not very convincing. In his account I find no evidence that the things which he depicts are really amoebae. They might equally well have been cells.

It may be added that two different amoebae may really occur occasionally in the sputum—*E. gingivalis*, from the mouth, and *E. histolytica*, from a lung abscess or a liver abscess which has ruptured into the lung.

"*Entamoeba undulans* Castellani, 1905." — Castellani (1905), has

described an "amoeba" from the intestine of man and named it "*E. undulans*." From his description and figures there can be no doubt that these "amoebae"—which possessed an undulating membrane—were really degenerate forms of *Trichomonas hominis*, with which his patient was said to be also infected. This has already been pointed out by a number of workers (Wenyon (1913), Hartmann (1913), etc.). The non-flagellate, amoeboid, degenerating forms of various species of *Trichomonas* are now familiar to all workers who have studied these organisms. It may be noted that they were probably first mistaken for amoebae in man by Lambl (1860); and that they were seen and first correctly identified as degenerating flagellates* by Cunningham (1871). Since then they have many times been mistaken for amoebae.

"*Craigia*."—Craig (1906) has described a remarkable organism to which he originally gave the name *Paramoeba hominis*—believing it to be similar to the marine *Paramoeba eilhardi* Schaudinn.† Subsequently it was placed in the new genus *Craigia* by Calkins (1912). The organism is said to live in the intestine, to be pathogenic, and to possess both amoeboid and flagellate stages in its life-history. The amoeboid forms apparently resemble *E. coli* and measure 10-25 μ in diameter. They form multinucleate cysts which liberate broods of flagellates "10-20 μ in diameter." Although Craig has published two accounts of this organism (1906, 1910), and made frequent reference to it in other works, he has never advanced adequate evidence for its existence. To prove that an organism such as he postulates really exists, requires further evidence,—evidence, moreover, of quite a different order from anything which he has yet been able to adduce. There is, nevertheless, abundant evidence in Craig's works that he was not, when he wrote, sufficiently familiar with the common intestinal amoebae and flagellates of man to be able to distinguish a new organism of the type described—supposing it to exist. Obvious sources of error and confusion were not excluded; and in the absence of all essential information concerning the cytology of "*Craigia*," I am unable to accept his conclusions.

So far as I am aware "*Craigia*" has been found subsequently by only one other worker—Barlow (1915). This writer not only "confirmed" Craig's observations but extended them by discovering a new species. According to him, there are really two species of "*Craigia*,"—*C. hominis* and *C. migrans*. His descriptions of both, however, serve only to confirm my suspicions that all these organisms are in reality a mixture of other and more familiar species of amoebae and flagellates. I have never found any organism which resembles *Craigia*: and until it has been vouched for by some independent and competent protozoologist—familiar with all the intestinal protozoa of man, and in possession of first-rate preparations of all the stages described—I am unable to believe in its existence. The name *Craigia* should therefore, in my opinion, be regarded as a *nomen nudum*, or as a partial synonym of at least two other

* Cunningham, however, called the organism "Cercomonad A," and was not aware that it was a *Trichomonas*.

† I am inclined to believe that *P. eilhardi* is itself probably a fictitious organism, formed by combination of a rhizopod with the swarmers of an alga. I find Schaudinn's account of it far from convincing.

generic names (a flagellate and an amoeba), until adequate evidence can be produced in favour of its retention.

"*Amoeba pyogenes* Verdun & Bruyant, 1907."—This name has been given to an amoeba found in the pus from an abscess in the malar region by Verdun and Bruyant (1907, 1907 *a*). It is said to be actively motile and to measure 20-35 μ in diameter, with a nucleus of 8-15 μ; and to form cysts measuring 6-15 μ and containing from one to four nuclei. The endoplasm is described as granular, and filled with digestive vacuoles containing red blood corpuscles and leucocytes. According to its describers the species resembles "*Amoeba coli* Lösch" (*i.e.*, *E. histolytica*), and the organisms found in similar situations by Kartulis, Flexner, and others (*i.e.*, probably *E. gingivalis*).

In my opinion this species was probably a mixture of *E. gingivalis* and cells of various sorts. At present there is insufficient evidence for regarding it as new. Smith and Barrett (1915, 1915 *a*), however, regard it as probably an independent species—related to, or possibly identical with, some very questionable "amoebae" found by Ribbert in Stensen's duct. They also suggest that it has affinities with "*E. mortinatalium*," mentioned below.

The Amoebae described by McCarrison (1909).—I have already identified two of the intestinal amoebae studied by McCarrison (1909). His "Amoeba I" was certainly, as he supposed, *E. coli*. His "Amoeba II" was equally certainly a name given to cells from the intestine—not to *E. histolytica* as he surmised. It only remains to add that the "third amoeboid body" which he briefly described and figured was undoubtedly the flagellate *Giardia* (=*Lamblia*) *intestinalis*, and therefore not an intestinal amoeba at all.

"*Endamoeba mortinatalium* Smith & Weidman, 1910."—Smith and Weidman described, in 1910,* some "amoebae" which they found in the kidneys, liver, and lungs of a still-born foetus. They subsequently described another similar "infection" in a 2-months old syphilitic child (Smith and Weidman, 1914). The amoebae in both cases were believed to be of the same species, for which the name *E. mortinatalium* was proposed. Smith and Weidman (1914) state that similar "protozoa" had been found in the kidneys of a syphilitic newborn infant by Ribbert, and later by the same worker in the parotid glands of non-syphilitic children; and that Jesionek and Kiolemenoglou found similar bodies in the kidneys, liver, and lungs of a syphilitic foetus. Smith and Weidman consider their "amoebae" to be identical "with those in at least Ribbert's first case and in that of Jesionek and Kiolemenoglou."

"*E. mortinatalium*" is described as an amoeba measuring usually 22-30 μ in diameter. Its nucleus is from one third to one half the diameter of the whole organism, and contains a large central karyosome. It should be noted that the "organism" was never seen alive, so that although "pseudopodia" are mentioned there is no evidence of its motility. From the figures and description I am entirely at a loss to know why the bodies in question are regarded as amoebae at all.

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* According to Smith and Weidman (1914) their first paper was published in the *University of Pennsylvania Medical Bulletin*, 1910, but I have not been able to consult it.

To my mind it seems probable that they are merely large cells in the organs studied. It seems premature to discuss the systematic position of this amoeba, therefore, before we know that it really is one.

It is difficult to understand how infection takes place if "*E. mortinatalium*" really is an amoeba. Smith and Weidman (1914) consider that in both their cases the mother was primarily infected, and the child acquired its infection "from some as yet unknown focus in her." "Doubtless," they say, "the parasites are harmless for the adult mother; while for the fetus, especially when impaired by luetic taint, they may well prove pathogenic and capable of destroying life." Doubtless: but one would like some evidence before accepting these speculations as facts. For the present it will suffice to notice that "*E. mortinatalium*" differs radically from all species of amoebae proved to occur in man.

"*Entamoeba polecki* Prowazek, 1912."—Most of the species of *Entamoeba* described at various times by Prowazek have already been identified: but there remains one of his species which has not yet been mentioned. This is an organism—or organisms—which he named "*Entamoeba polecki*" (Prowazek, 1912). It has been justly remarked by James (1914) that "this writer worked with very insufficient material": and the present species forms no exception. James, indeed, even doubts whether the name "*E. polecki*" was really given to "entamoebae or something else."

In a very brief note Prowazek (1912) states that he found the organism in a pig, and also in the stools of a child, in Saipan (Ladrone Islands). It is said to be "10-12 μ large"; and it is stated further that in "older faeces" amoebae measuring only 5 μ were also observed, which copulate in pairs and encyst. A few figures are given, but they are difficult to interpret. There is nothing to show that this "species" is not a mixture of amoebae* from pigs and human beings confused with free-living species from stale human stools.

In view of the astonishing habitat and development ascribed to this organism, the absence of evidence in support of Prowazek's statements, and the fact that no recognizable description has yet been published, I consider that "*E. polecki*" is a species which should be rejected.

"*Entamoeba dysenteriae europeae* Popper, 1917."—Under this name another "new amoeba" has recently been described by Popper (1917). It was found in the stools of patients suffering from dysentery in Galicia and Hungary. Although it is described at length, it is impossible to recognize, in the description, anything which greatly resembles an amoeba. Most of the cytological characters necessary for its determination are not recorded; and the account shows such obvious ignorance of amoebae generally, and of the intestinal amoebae of man in particular, that it can hardly be doubted that Popper's "new amoeba" is really a mixture of cells—and possibly other bodies—from dysenteric stools. I shall not discuss this "organism" further. But I may note the following as samples of the author's observations, and in justification of my rejection of this species. "*E. dysenteriae europeae*" is a "colourless cell"

* I note that Brumpt (1913) and Pestana (1917) regard "*E. polecki*" as a synonym of *E. coli*, and that Hartmann (1913) retains it as an independent species. Neither of these views appears to me justifiable.

filled with "numerous granules" showing great activity caused by "protoplasmic streaming." Its size is very variable, but is "estimated" at "30 μ and more." The nucleus is large and vesicular or kidney-shaped. The "amoeba" forms bud-like "pseudopodia" very rapidly, but does not undergo locomotion. The "cysts" are "large," "without distinctly recognizable membrane." Their contents were not investigated, but they are said to have been tightly packed with highly refractile masses. It is said, finally, to resemble "Amoeba coli Loesch" and to differ from "Amoeba histolytica Schaudinn"—which are, of course, two different names for the same organism.

There is thus every justification for removing this improperly named and probably non-existent "amoeba" from among the amoebae of man and the causes of dysentery.

The Amoebae described from Human Skin Lesions.—It has already been noted that amoebae have been described from the skin. Carini (1912, 1912*a*) has recorded two cases in which a phagedaenic ulceration developed in the tissues surrounding the wound made in operating upon a liver abscess. He found numerous amoebae in the subcutaneous tissues and in the exudate from the ulcers; and he regarded them as belonging to *E. histolytica*—apparently believing that they invaded the skin secondarily from the pus draining from the liver abscess. Dagorn and Heymann (1912) have described a similar case, and a report upon the amoebae found has been published by Gauducheau (1912*a*). Several other similar cases are also on record. The first appears to be that of Nasse (1892), who did not see the amoebae alive, and whose material was admittedly badly fixed and prepared.

It is, I think, still very doubtful, from the descriptions published, whether the "amoebae" found in such phagedaenic lesions really were *E. histolytica*. No proper account of them has yet been published, and no protozoologist with an adequate systematic knowledge of the amoebae appears to have studied any of these cases. Although Carini merely says* that his amoebae had affinities with "*Amoeba tetragena*," he appears to assume their identity with this species (*i.e.*, *Entamoeba histolytica*). He has given no proper description of them. Gauducheau (1912) gives an account of his "amoebae" which leads one to doubt whether they were amoebae at all. He did not attempt to identify them, because he holds—if I understand him correctly—that it is not possible to distinguish different species of amoebae from one another. It thus seems clear that further investigations by a competent protozoologist are necessary; and until more information is available, it seems to me unprofitable to discuss the nature of these "amoebae."

In the same category may be placed the "amoebae" found by J. L. Maxwell (1912) is cases of "fistulous disease of the buttocks" observed in Formosa. Although the author states it as his opinion that the pus from the fistulous tracks contained "amoebae, conforming I believe to the type of *entamoeba histolytica*," and would regard these as the cause of the condition, there seems at present to be no evidence of any weight in support of such an interpretation. The exact systematic position of

* "Les amibes présentent des caractères les rapprochant de l'*Amoeba tetragena*" (Carini, 1912).

these "amoebae" can hardly be discussed before it has been shown that they really are amoebae.

Many other amoeboid organisms have been described from man. In fact there is hardly an organ in the body from which somebody, at some time, has not obtained "amoebae." In this respect the amoebae have been maltreated in much the same manner as the coccidia, and it would not be difficult to compile a long list of "pseudo-amoebae" from medical literature. But further discussion of these appears to be unnecessary; and it seems best to leave them in that oblivion into which they have, for the most part, justly fallen.

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PLATE I.

All the figures in this plate are drawn from preparations fixed with sublimate-alcohol (with or without acetic acid), and stained by Mann's methylblue-eosin method (modified), unless a different technique is indicated. Magnification 2,500 diameters.

Figs. 1—6. *Entamoeba histolytica.*

Fig. 1. *E. histolytica*, active amoeba scraped from an intestinal ulcer (cat), showing typical structure of nucleus, and partly digested remains of three ingested red corpuscles. (Fixed in Bouin's fluid.)

Fig. 2. Precystic amoeba, from human stool (subacute amoebic dysentery).

Figs. 3, 4, 5. Uninucleate, binucleate, and quadrinucleate cysts respectively: from same case as preceding. Strain forming cysts (living) with mean diameter of ca. 13·5 μ. Note nuclei, chromatoid bodies (red), and clear spaces in 3 and 4 representing glycogen vacuoles.

Fig. 6. Binucleate cyst showing glycogen (red patch). Fixed with Carnoy's fluid, stained with Best's carmine.

Figs. 7, 8, 9. *Endolimax nana.*

Fig. 7. Free *E. nana*, in human stool. Note nucleus, ingested bacteria, pseudopodium, etc.

Fig. 8. Mature 4-nucleate cyst of *E. nana*, from same case.

Fig. 9. Binucleate cyst, containing glycogen mass (red). Fixed in sublimate-alcohol, stained with Best's carmine.

Figs. 10, 11. *Iodamoeba bütschlii.*

Fig. 10. Free *I. bütschlii*, in human stool. Note nucleus, ingested bacteria, etc.

Fig. 11. Mature cyst. Note nucleus, volutin granules (pink), and clear space occupied by glycogen mass in living cyst ("I. body").

Figs. 12—15. *Entamoeba coli.*

Fig. 12. Active amoeba, in human stool. Note structure of nucleus, ingested bacteria, etc. (and compare with fig. 1).

Fig. 13. Precystic amoeba, from same case. Note nuclear structure, absence of ingested food, etc. (and compare with fig. 2).

Fig. 14. Mature 8-nucleate cyst, from same case. Note nuclear structure (compare with figs. 12 and 13, and figs. 1—5), and small chromatoid body (red).

Fig. 15. Binucleate cyst, containing large glycogen mass (red). Fixed in Carnoy's fluid, stained with Best's carmine. (Cf. figs. 6 and 9.)

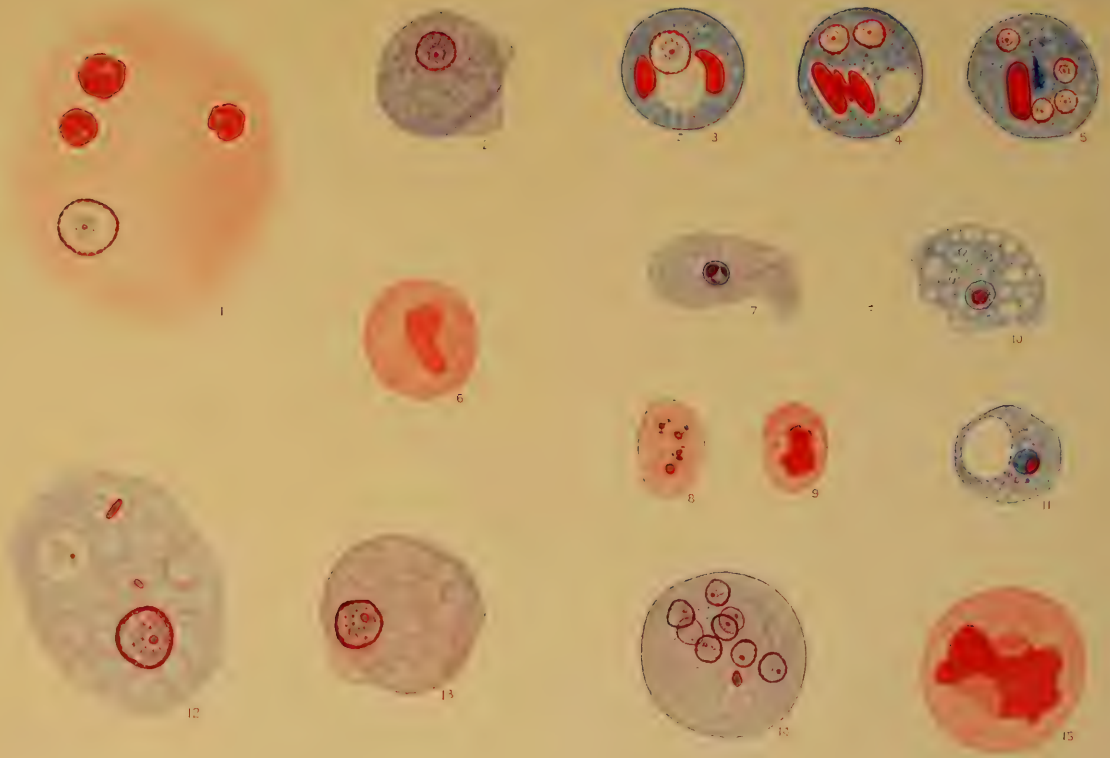

PLATE II.

All figures—unless otherwise stated—from specimens fixed in sublimate-alcohol and stained with Heidenhain's iron-haematoxylin and eosin. Magnification 2,500 diameters.

Fig. 16. *Entamoeba histolytica.* Large active amoeba, containing three red blood-corpuscles, from stool of a case of amoebic dysentery (human). Stained with Weigert's iron-haematoxylin and eosin.

Fig. 17. *Entamoeba coli.* Large active amoeba, from human stool. Observe the nucleus, ingested bacteria and other food-inclusions in endoplasm, etc. (Compare with fig. 16.)

Figs. 18—31. *Endolimax nana.*

Figs. 18—23. Active amoeboid forms of *E. nana*, from human stools: showing various forms of nuclear structure, etc.

Fig. 24. Eight nuclei from other individuals of *E. nana*, to show some of the commoner types of karyosome seen in this species.

Figs. 25, 26, 27. Uninucleate, binucleate, and quadrinucleate (mature) cysts respectively.

Figs. 28, 29. Cysts (mature) of *E. nana* with filamentar and granular inclusions.

Fig. 30. Very small mature cyst.

Fig. 31. Unusually large cyst.

Figs. 32—42. *Iodamoeba bütschlii.*

Figs. 32—34. Active *I. bütschlii* amoebae from human faeces. Note nuclear structure, ingested micro-organisms, etc. (Compare with figs. 16—23.)

Figs. 35, 36. Precystic amoebae. Observe the changes taking place in nuclear structure, freedom from cytoplasmic inclusions, etc.

Fig. 37. Organism just encysted. Note structure of nucleus (compare with preceding figures), volutin granules (pink) in cytoplasm, and small clear space representing glycogen mass in living organism.

Fig. 38. A large cyst, badly fixed, and showing much protoplasmic shrinkage, etc. [For comparison with the figure of the cyst of "*Entamoeba*" *bütschlii* published by Prowazek (1912*a*, Pl. XVIII, fig. 21).]

Fig. 39. Mature cyst, containing large glycogen mass (red). Fixed in Carnoy's fluid, stained with Weigert's iron-haematoxylin and Best's carmine.

Figs. 40, 41, 42. Mature cysts, showing typical structure of nucleus, volutin granules, and glycogen "vacuole" (represented as a clear space after removal of the glycogen). 41 and 42 are cysts of irregular shape, such as are often formed by this species. (The irregular outlines are not artificially produced by the cytological reagents used.) Stained with haematum and eosin.

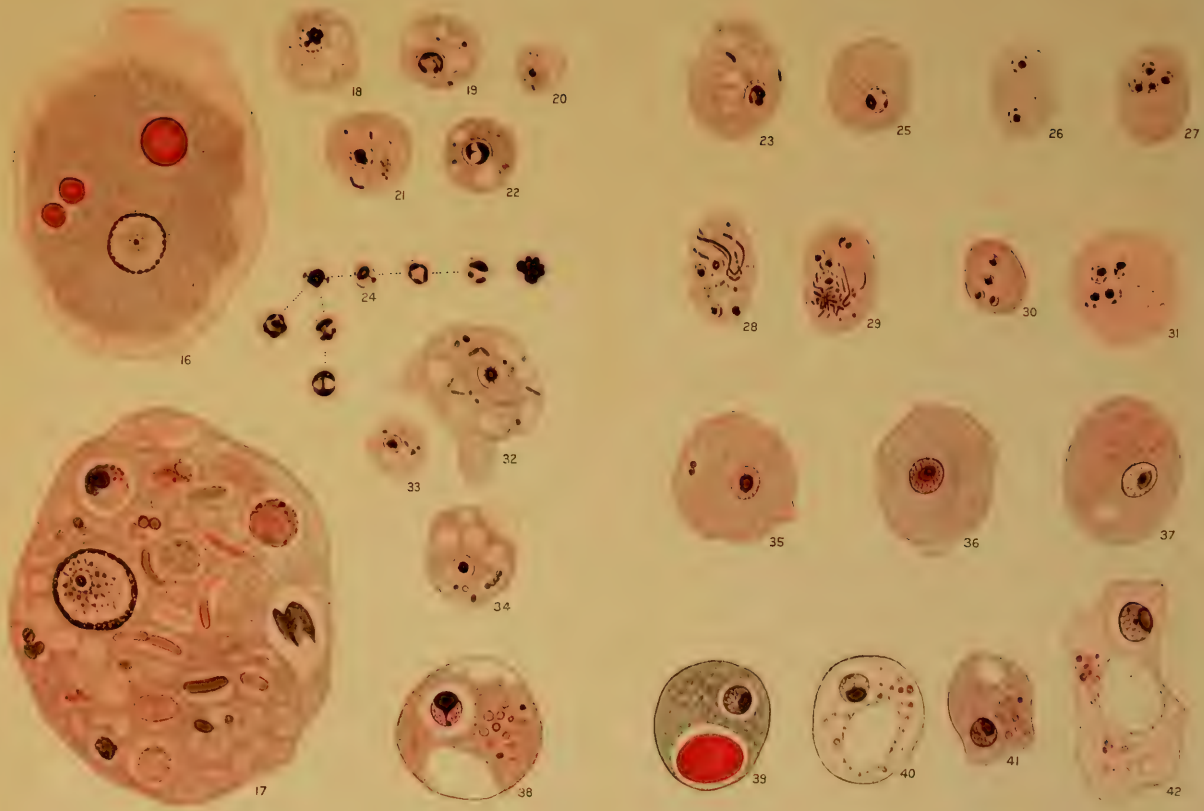

PLATE III.

All drawings made from amoebae in sections of intestinal ulcers of kittens experimentally infected with *Entamoeba histolytica*. All material fixed with Bouin's fluid, and sections stained as follows:

Figs. 43, 44, 48. Heidenhain's iron-haematoxylin, fuchsin S, and picro-indigo-carmine.

Figs. 45, 46, 49. Fuchsin S, picro-indigo-carmine.

Fig. 47, Heidenhain's iron-haematoxylin, orange G.

Figs. 50, 51. Mann's methylblue-eosin (modified).

Figs. 52, 53. Safranin and light green.

Fig. 54. Borrel's stain (magenta and picro-indigo-carmine).

The drawings show successive stages in the normal process of division in *E. histolytica*. For fuller description see text, pp. 41-43. (The dark bodies in the cytoplasm are remains of ingested red corpuscles.) Magnification 2,500 diameters.

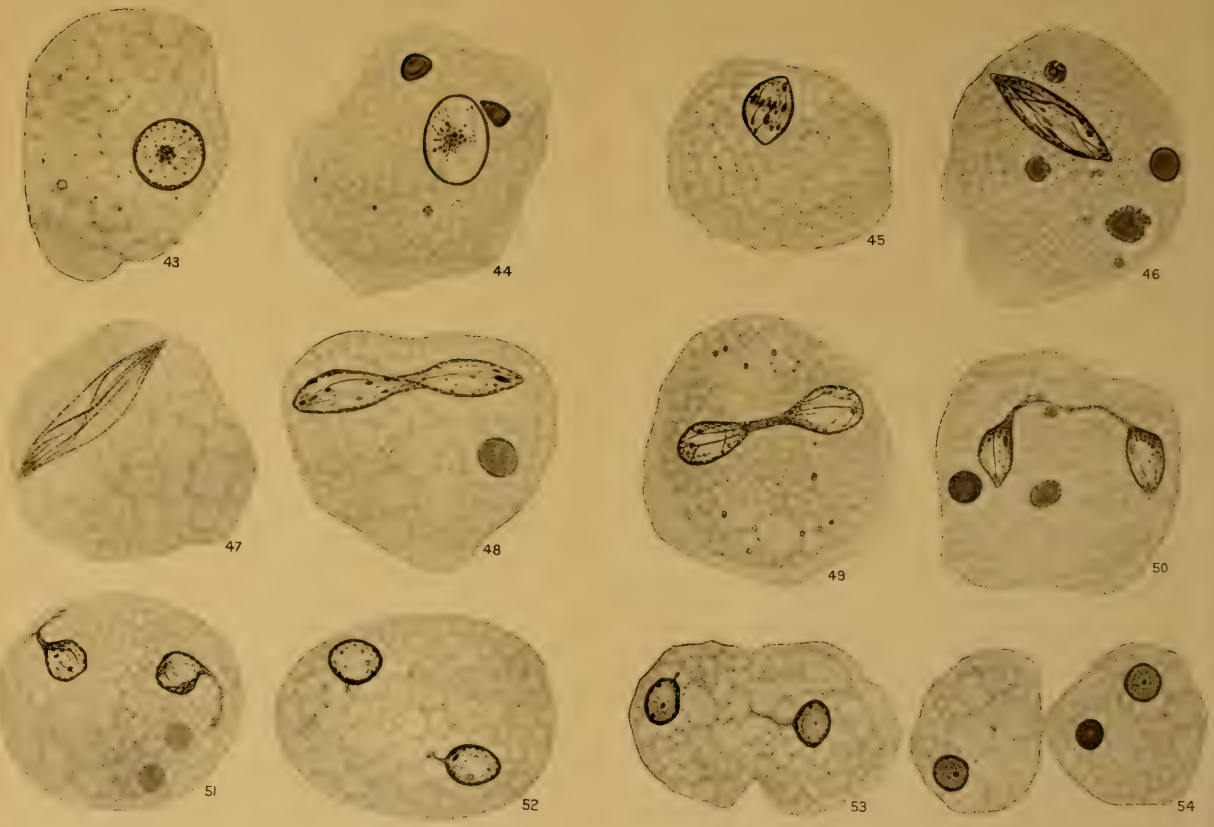

PLATE IV.

All figures drawn—unless otherwise stated—from specimens fixed in sublimate-alcohol and stained as indicated. Magnification 2,500 diameters.

Figs. 55—69. *Entamoeba coli.*

Fig. 55. Active *E. coli* from human stool, showing "clefts" in cytoplasm, etc. (Weigert's iron-haematoxylin.)

Figs. 56—69. Cysts of *E. coli* at various stages in development.

Fig. 56. Newly-formed uninucleate cyst, showing minute chromatoid bodies, glycogen space, etc. (Stained haemalum.)

Fig. 57. Later cyst, with very large nucleus preparing for first division. Note chromatoid bodies and glycogen "vacuole." (Mann's stain, modified.)

Fig. 58. First nuclear spindle. (Heidenhain's iron-haematoxylin and eosin.)

Figs. 59, 60. Binucleate cysts. Note glycogen "vacuoles," chromatoid bodies, etc. (Compare with fig. 15, Pl. I.) Fig. 59 from cyst stained by Mann's method (modified): Fig. 60, haemalum.

Fig. 61. Quadrinucleate cyst. Note that all nuclei are in early stages of division—as they usually are in 4-nucleate cysts of this species. Fixed Bouin's fluid, stained alcoholic ferric-chloride iron-haematein.

Fig. 62. Typical 8-nucleate cyst (mature). Note structure of resting nuclei. (Heidenhain's iron-haematoxylin.)

Figs. 63—66. *E. coli* cysts, at various stages of development, containing chromatoid bodies of different forms. Fig. 65 shows a mature cyst with filamentar chromatoids—probably a cyst of the type attributed by Prowazek to "*Entamoeba williamsi.*" (All these cysts stained with haemalum, progressively.)

Fig. 67. Abnormal 16-nucleate cyst of *E. coli.* (Stained with haemalum.)

Fig. 68. Very small 8-nucleate cyst of *E. coli.* (Haemalum and eosin.)

Fig. 69. Very small 4-nucleate cyst of *E. coli*, containing 4 resting nuclei and chromatoid bodies. Compare with fig. 71, etc. (Haemalum and eosin.)

Figs. 70—76. *Entamoeba histolytica.*

Fig. 70. Cyst of *E. histolytica* (uninucleate), containing very abundant chromatoid bodies. (Fixed alcoholic picro-acetic-acid, stained paracarmine.)

Fig. 71. Mature 4-nucleate cyst, without chromatoid bodies. (Haemalum.) Compare with fig. 69.

Figs. 72, 73, 74. Three very small cysts of *E. histolytica*—uninucleate, binucleate, and quadrinucleate respectively—belonging to a strain producing cysts (living) with an average diameter of 6·6 μ. (Haemalum.)

Figs. 75, 76. Two very large cysts of *E. histolytica*—respectively uninucleate and quadrinucleate—belonging to a strain forming (living) cysts with an average diameter of 15 μ. (Haemalum.) Compare with preceding figs. 72-74, and with figs. of *E. coli* on this plate.

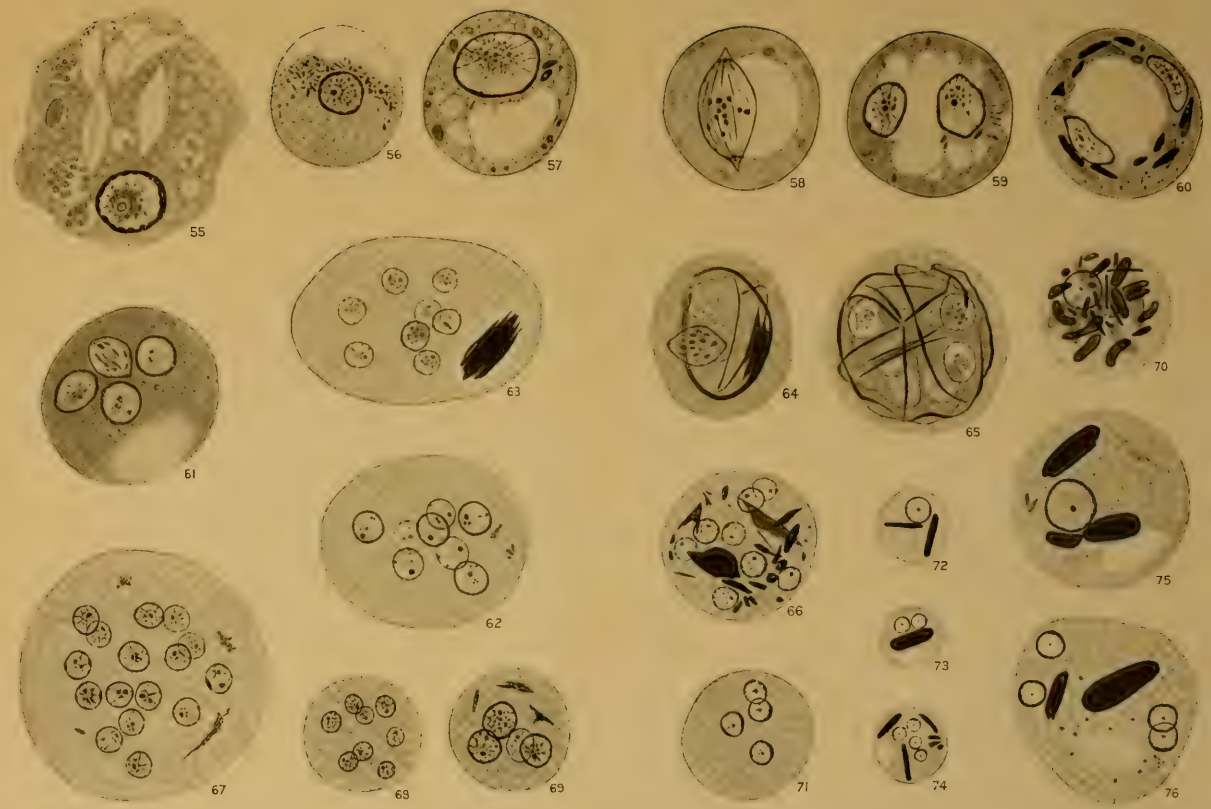

PLATE V.

All figures drawn from preparations fixed with sublimate-alcohol and stained with Heidenhain's iron-haematoxylin unless otherwise indicated. Magnification 2,500 diameters.

Figs. 77—85. *Entamoeba histolytica.*

Figs. 77, 78. Precystic forms of *E. histolytica* belonging to strains producing small cysts—such as those shown in figs. 72—74, Pl. IV. (Stained with haemalum.)

Figs. 79, 80. Precystic amoebae belonging to strains forming cysts of larger size—such as those shown in figs. 3—5, Pl. I.

Fig. 81. Precystic amoeba from a stale stool. Degenerate and invaded by bacteria. Compare nucleus with those in figs. 77—80.

Fig. 82. Precystic amoeba with rod-like chromatoid bodies—resembling ingested bacteria. (Haemalum.)

Fig 83. Precystic amoeba parasitized by bacteria. (Alcoholic iron-haematein.)

Fig. 84. Degenerate uninucleate cyst, belonging to same strain—also parasitized by bacteria. (Alcoholic iron-haematein.)

Fig. 85. Degenerate uninucleate cyst from another infection. (Weigert's iron-haematoxylin.) The cyst contains 3 chromatoid bodies, and a number of parasitic bacteria, like those in figs. 83 and 84. (For fuller description of these forms see text, p. 63 *et seq.*)

Figs. 86—89. *Endolimax nana.*

Fig. 86. Abnormal 8-nucleate cyst of *E. nana.* (Haemalum.) Compare with figs. 27, 30, 31, Pl. II.

Figs. 87, 88, 89. Individuals of *E. nana* parasitized by *Sphaerita* sp. Figs. 87, 88, from specimens stained with haemalum: showing respectively an amoeba containing two sporangia, and one with a single spore-morula and younger stages. Fig. 89, specimen stained with iron-haematoxylin, containing one large and one small spore-morula.

Figs. 90—92. *Dientamoeba fragilis.*

Figs. 90, 91. Two ordinary binucleate individuals from human stools.

Fig. 92. A uninucleate specimen.

Figs. 93, 94. *Entamoeba gingivalis.*

Fig. 93. An individual with various cytoplasmic inclusions, and a nucleus with an eccentric karyosome. From human mouth. (Case of pyorrhoea alveolaris.)

Fig. 94. Similar organism. Nucleus with central karyosome. (Compare with figs. 77—80 on this Plate, and figs. of *E. coli* and *E. histolytica* on Plate I.)